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Enhanced killing of intracellular multidrug-resistant *Mycobacterium tuberculosis* by compounds that affect the activity of efflux pumps

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Whereas human neutrophils are effective and efficient killers of bacteria, macrophages such as those derived from monocytes are almost devoid of killing activity. Nevertheless, monocytes can be transformed into effective killers of mycobacteria or staphylococci when exposed to clinical concentrations of a phenothiazine or to inhibitors of efflux pumps (reserpine and verapamil), or to ouabain, an inhibitor of K^+ transport. Because the rates of multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) continue to escalate globally, and because no new effective drug has been made available for almost 40 years, compounds that enhance the killing activity of monocytes against MDR-TB are obviously needed. This review covers the specific characteristics of MDR-TB, identifies a variety of agents that address these characteristics and therefore have potential for managing MDR-TB. Because the mechanism by which these agents enhance the killing of intracellular bacteria is important for the intelligent design of new anti-tubercular agents, the review correlates the mechanisms by which these agents manifest their effects. Lastly, a model is presented which describes the mechanisms by which distinct efflux pumps of the phagosome–lysosome complex are inhibited by agents that are known to inhibit K^+ flux. The model also predicts the existence of a K^+ activated exchange (pump) that is probably located in the membrane that delineates the lysosome. This putative pump, which is immune to inhibitors of K+ flux, is identified as being the cause for the acidification of the lysosome thereby activating its hydrolytic enzymes. Because the non-killer macrophage can be transformed into an effective killer by a variety of compounds that inhibit K^+ transport, perhaps it would be wise to develop drugs that enhance the killing activity of these cells inasmuch as this approach would not be subject to any resistance, as is the eventual case for conventional antibiotics.

Keywords: phenothiazines, MDR-TB, killing activity

Introduction

Mycobacterium tuberculosis has infected man since the birth of civilization.[1,2] This long association has in all probability been the selection process by which this organism evolved into a steadfast human pathogen. The steadfast nature of this human infection has been the basis for its eventual elimination as a human pathogen inasmuch as the effectiveness of therapy with antibiotics available in the 1950s was readily proven by the steady decline of new cases of pulmonary tuberculosis throughout the Western World.[3,4] With respect to Third World countries, infections continued to increase due to man-made conditions such as famine, war and over-crowding, as well as to the cost of anti-tubercular drugs, which for many countries was beyond their affordability.[5] The movement of large numbers of people (refugees) from Third World countries that had experienced war, political strife or famine, to parts of the West, contributed decades later, to significant increases of new cases of pulmonary tuberculosis. Although these new cases of pulmonary tuberculosis were initially restricted to these immigrants,[6–8] the infection began to spread rapidly among the indigenous population of the cities where the immigrants had settled.[8] Despite the availability of effective anti-tubercular compounds, new cases of pulmonary tuberculosis continued to escalate, and in some cities such as New York, the rate of new cases was extremely alarming.[9,10] One of the reasons for this escalation involved the appearance of large numbers of cases that were infected with *M. tuberculosis* that was resistant to the two most effective anti-tubercular drugs, isoniazid and rifampicin;[11] hence, patients infected with multidrug-resistant *M. tuberculosis* (MDR-TB) strains were sources for new transmissions of infections, which rapidly manifested themselves as active disease in patients co-infected with HIV.[12] Moreover, because of the availability of anti-tubercular drugs and ineffective therapy, strains initially susceptible to

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isoniazid and rifampicin could infect another patient who, due to the selection of a spontaneous mutation that resulted in resistance to one antibiotic, would be managed poorly with these agents, and a second spontaneous mutation that caused resistance to the second antibiotic would be selected.[13] Therapy of MDR-TB is extremely problematic, regardless of whether one uses all five first-line drugs of defence (isoniazid, rifampicin, streptomycin, ethambutol, pyrazinamide), or second line of defence compounds.[14] Furthermore the use of these therapeutic modalities produces significant morbidity in view of the problem of non-compliance.[15] Hence new cases of MDR-TB have continued to increase in many of the urban centres of Western and Third World countries.[16]

Tuberculosis: the disease and requirements for an effective anti-TB drug

Mycobacterium tuberculosis infects the lung parenchyma intracellularly and generally remains silent for 90% of those infected, and for many of these cases, the infection is resolved, and for still fewer cases, evidence of infection is noted only at autopsy. When the organism breaks free of its intracellular prison in large numbers it manifests its presence by dissemination to other sites of the lung, thereby increasing the size of the area infected. At this time the organism may be expelled via microdroplets of sputum to the environment when the patient coughs. When this occurs the infection has progressed to one of active disease. At this stage the patient is infectious. The percentage of infected patients that progress to active disease can be drastically increased by conditions that promote immuno-incompetence.[17–24]

An effective anti-tubercular drug must satisfy two conditions—namely, it must be able to inhibit the replication of *M. tuberculosis* at its intracellular location and/or kill the organism directly at that site.[15,25,26] Surprisingly, with few exceptions, almost all studies that report on the anti-tubercular activity of old or new drugs have been conducted *in vitro*. Subsequent to these studies, the compounds are then tested in animal models for their ability to cure or reduce a tuberculosis infection. Because very few, if any, show any effectiveness on the infected mouse, it is not surprising that few of these compounds reach clinical trials, and even fewer may make it to the market place. For this reason the last effective anti-TB compound that reached the market was rifampicin, almost 40 years ago.[27] Why is there such a discrepancy between the many drugs that demonstrate activity *in vitro* and fail to cure a TB infection *in vivo*? This is probably due to their inability to reach the intracellular organism or maintain activity at that site.[28,29]

The phenothiazines: compounds that satisfy the two essential aspects of an effective anti-tubercular drug

The antimicrobial activity of phenothiazines has been known for over a century.[26] The first phenothiazine to be examined for antibacterial properties was the dye methylene blue.[26,30] This dye (Figure 1) could render mobile bacteria immobile[31,32] as well as inhibit the *in vitro* growth of some Gram-positive bacteria.[33–35] However, soon after the demonstration of its antibacterial properties, the dye was shown to cause cats to become lethargic.[36] Interest in the neuroleptic properties of the dye overshadowed its antimicrobial properties—hence, the dye was used as a lead compound for the synthesis of the first neuroleptic, chlorpromazine, which is a colourless phenothiazine (Figure 1). The availability of chlorpromazine in 1957 resulted in its worldwide use for the therapy of psychoses and severe neuroses, through which it soon became clear that chlorpromazine had antibacterial properties, including those of an anti-tubercular drug.[37] Nevertheless, little interest in chlorpromazine, or any other phenothiazine, as an antibacterial drug (antibiotic) was generated, since it was the 'Golden Age of Antibiotics'[38] and the drugs available at that time (1960s) were shown to be very effective at controlling tuberculosis, given the rapid and steady decline of the disease.[39] However, with the extensive use of antibiotics, the problem of resistance began to appear in the early 1960s[40] and escalated to such significant levels that for many bacterial infections, treatment became problematic.[41–44] Although the response of the pharmaceutical industry kept pace initially with the problem, by rapidly making available new antibiotics, the time between the introduction of a given antibiotic and ensuing significant rates of resistance grew shorter.[45–48] By the 1990s, antibiotic resistance became the 'norm' for many bacterial infections; with respect to *M. tuberculosis* this was to rifampicin and isoniazid, the two most effective anti-tubercular compounds. MDR-TB was significant in many areas of the world, including urban centres in Western Europe (e.g. Lisbon, Barcelona), and in Northern Europe (e.g. Riga).[49] The failure of conventional anti-tubercular therapy experienced early in the 1990s spurred a search for new anti-tubercular drugs—a search that has thus far resulted in no new compounds that are as effective as those to which resistance had developed. Because the problem of MDR-TB primarily took place in countries that were economically disadvantaged,[27,28] the required incentive was not present for the creation of new and effective compounds, given the high cost of drug development and the poor market conditions present in the countries affected. Prior to and during the emergence of MDR-TB, phenothiazines had been observed to have potential for the therapy of tuberculosis.[25] The studies described in that review indicated that the administration of chlorpromazine to patients presenting with tuberculosis resulted

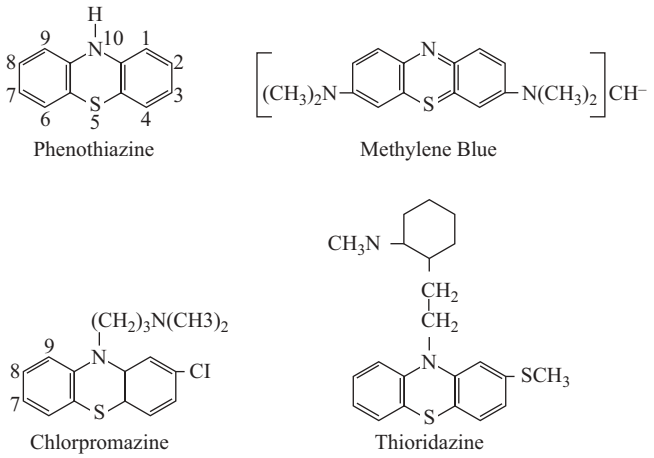

Figure 1. Methylene blue, chlorpromazine, thioridazine and general phenothiazine structures.

in cures.[25] The ability of chlorpromazine to cure tuberculosis resulted in a series of *in vitro* studies that not only showed that chlorpromazine was an effective anti-mycobacterial compound,[50–54] but also that other phenothiazines (see Figure 1) were equally effective.[51,55–62] Nevertheless, the concentrations of chlorpromazine and other phenothiazines required for *in vitro* inhibition of mycobacterial growth were well beyond those that could be achieved in the patient.[25,26] However, because phenothiazines were known to be concentrated by tissues and organs containing large populations of macrophages,[63–65] Crowle *et al.* demonstrated that physiological concentrations of chlorpromazine present in the medium could enhance the killing of *M. tuberculosis* that had been phagocytosed by macrophages.[50] Because chronic administration of chlorpromazine is known to produce a wide range of mild-to-severe side effects, the use of this compound for the therapy of tuberculosis was not seriously considered.[25,26] Given the fact that phenothiazines are concentrated by macrophages and other cells rich in lysosomes,[66–72] thioridazine (Figure 1), being equivalent to chlorpromazine in all of its anti-mycobacterial properties,[25,26,53,54] was studied for its ability to enhance the killing of phagocytosed bacteria, including *M. tuberculosis*.[73–75] This series of *ex vivo* studies demonstrated what had been previously observed for chlorpromazine,[50] namely that concentrations in the medium that were below those present in the plasma of patients chronically treated with thioridazine could result in the killing of intracellular mycobacteria.[73] These results led the way for studies that would determine whether phenothiazines could reduce bacterial load of the lung in mice infected with *M. tuberculosis*.

That thioridazine can effectively reduce that load is shown by the preliminary study summarized in Figure 2. However, because the manner of infection involved massive intraperitoneal doses of *M tuberculosis*, whereas the number of organisms of the pulmonary system could be effectively reduced, those present in the spleen and liver were relatively unaffected. Hence, the possibility of vertical transmission of *M. tuberculosis* to the lungs or other organs remained a distinct possibility. Since that demonstration, others have shown that derivatives of chlorpromazine could also reduce the number of organisms recovered from the lungs of the infected mouse.[76,77] Other phenothiazines have also been suggested for the management of tuberculosis.[57,58,61]

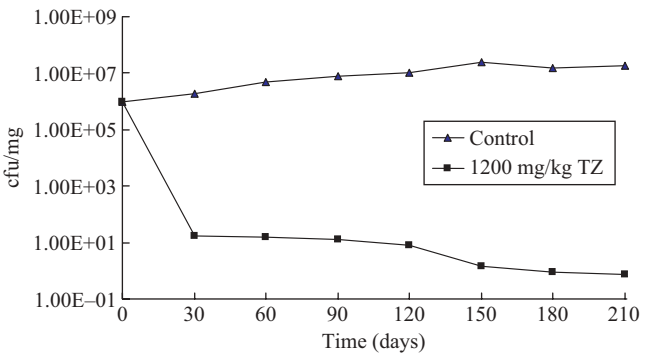

Figure 2. Effect of thioridazine (TZ) on the recovery of *M. tuberculosis* from infected mice. Four groups, each consisting of eight female mice were infected intraperitoneally with approx. 10^6 cfu of *M. tuberculosis* H37Rv ATCC 27294 and treated 3 days later with daily doses of thioridazine (100, 400 or 1200 mg/kg). The fourth group received no drug. At intervals of 30 days one mouse from each of the four groups was sacrificed, their lungs removed, weighed and homogenized, and mycobacteria were freed from cellular debris with the use of NaOH/sodium citrate/cysteine. Following centrifugation to remove cell debris, the supernatant was used for the preparation of serial dilutions required for colony-forming unit determination. Because treatment with daily doses of 100 and 400 mg/kg produced marginal results, these data are not shown. Controls, filled triangles; 1200 mg/kg, filled squares.

How does a phenothiazine enhance the killing of intracellular bacteria including mycobacteria?

Phenothiazines have been shown to inhibit the transport of K^+ from external to internal cellular compartments such as transport channels of cardiac Kir2.1 cells and red blood cells[78–80] and between intracellular compartments (e.g. diencephalic neurons, rat liver mitochondria).[81,82] They also inhibit the binding of calcium to calmodulin, the calcium binding protein of mammalian cells.[83] The binding of calcium to calmodulin-like proteins of bacteria has been amply demonstrated.[84–93] The inhibition of calcium access to Ca^{2+}-dependent ATPase inhibits transport processes such as those performed by influx and efflux pumps.[94–96] Because phenothiazines inhibit access to calcium, they inhibit the activity of calcium-dependent ATPase, and hence the transport processes.[94–101]

Bacteria as well as mammalian cells contain efflux pumps that extrude noxious agents from the periplasm and cytoplasm of the former[102] and from the cytoplasm of the latter.[103] Understanding the effects of a phenothiazine on calcium-dependent transport processes of the bacterium or the mammalian cell predict that their respective efflux pumps will be affected by a phenothiazine. This prediction has been shown to be correct whenever studied.[94–101] Since the first contact of phenothiazine with the bacterium takes place at the surface of the cell envelope, it would be expected that although the phenothiazine could penetrate this structure as a consequence of a concentration gradient and its amphipathic structure, one of the many efflux pumps of the bacterium would recognize this molecule and extrude it once it reached the periplasm of the bacterium. Although this expectation has yet to be fully studied, the effect of phenothiazine on the efflux system of the bacterial cell has been shown to be one of inhibition.[96–101] Phenothiazines readily intercalate between nucleic bases of the DNA helix.[104–108] The degree of intercalation is dependent upon the number of guanosine-cytosine residues.[105] When phenothiazines intercalate into DNA they inhibit all DNA-based processes as well as the degree of coiling and uncoiling of DNA promoted by gyrases.[109] The inhibition of the efflux pump by a phenothiazine would result in large numbers of phenothiazine molecules entering the cell, reaching their intercalative sites of the DNA and thereby inhibiting the replication of the bacterium.

The concentrations of the phenothiazine in the medium required for the inhibition of bacterial replication vary greatly depending on the type of bacterium: the MIC of phenothiazines for Gram-negative bacteria ranges from 100 to 200 mg/L, whereas for Gram-positive bacilli or cocci the MIC ranges from 10 to 40 mg/L.[26] Although not proven, there seems to be a strong correlation between bacteria that contain a highly effective system of efflux pumps and the concentration of the phenothiazine required for the *in vitro* inhibition of replication. As an example, as much as 35% of the genome of

Gram-negative bacteria codes for influx/efflux pumps (transporters) and, with respect to *Escherichia coli*, as many as 20 MDR efflux pumps have been genetically characterized.[102] Although this organism has an obvious redundancy of efflux pumps, the major efflux pump that accounts for MDR in this organism is coded by the *acrAB-TolC* operon.[97] When this operon is deleted, the organism becomes extremely susceptible to antibiotics, and to chlorpromazine and thioridazine.[97] When both the *acrAB*-intact and *acrAB*-deleted strains are induced to high levels of resistance to tetracycline, the genetic expression of the AcrAB efflux pump of the former has been increased 6-fold whereas the AcrEF efflux pump of the latter strain has been increased 80-fold.[97] The increased genetic expression of these pumps renders both strains significantly more resistant to chlorpromazine and thioridazine than their parents. These results support the contention that chlorpromazine and thioridazine are substrates of the AcrAB and AcrEF efflux pump systems and that when the ability of the pumps to extrude the agents is exceeded, the agents reach their intercalative targets and bacterial replication is inhibited. Because the concentration of either phenothiazine in the medium that enhances the killing of intracellular bacteria is about a 100th of that needed to kill the bacterium *in vitro*, [73–77] the killing activity appears to be related to the phenothiazine being concentrated by the non-killing macrophage to levels comparable to those required for killing *in vitro*.[50] Electron microscopic studies of the effects of phenothiazines on phagocytosed *Staphylococcus aureus* demonstrate that the *in vitro* effect of these agents on the bacterium's morphology is reproduced when the bacterium is phagocytosed by macrophages that are subsequently exposed to concentrations of the agent (see Figure 3) which produce neither an inhibition of replication nor a change in the morphology of the organism.[110]

Requirement of K^+ for intracellular killing

The killing activity of neutrophils, although highly complex, has been shown by a series of elegant studies conducted by Segal's group[111] to depend upon the availability of K^+ to the phagolysosome[112] and the dependence of this process on active Ca^{2+} channels of the phagolysosome unit. The essential need for Ca^{2+} required for the availability of K^+ involves a Ca^{2+}-dependent ATPase that is employed for the generation of energy required in K^+ transport. Because the K^+ concentration needed to trigger the acidification process required for the activation of hydrolases[113] is higher than that present in the cytoplasm[11,112] one would have to assume that the membrane of the phagolysosome complex would contain the required energy-driven efflux pumps. Because phenothiazines do transform non-killing macrophages into effective killers[50,73–75] and because phenothiazines are potent inhibitors of K^+ transport processes that are dependent upon Ca^{2+}-dependent ATPase, it seems probable that killing is enhanced by the phenothiazine's inhibition of K^+ efflux from the phagolysosome. If this hypothesis is correct, then one would predict that inhibitors of K^+ transport would also enhance the killing activity of non-killing macrophages. As is evident from Figure 4, ouabain, verapamil and reserpine, which are inhibitors of K^+ transport processes, also enhance the killing activity of non-killer macrophages.[114] The question of whether the K^+ efflux pump units pre-exist in the lysosome or are part of the phagosome unit, and hence have their origins in the plasma membrane of the macrophage, has not yet been addressed. Nevertheless, with the exception of smooth muscle,[115] the plasma membrane of most mammalian cells has a plethora of K^+ transport units.[116] In all likelihood the plasma membrane that delimits the phagosome probably contains many K^+ transport units whose origins are from the plasma membrane of the macrophage. Due to the invagination process that results in the phagosome, the plasma membrane would pump K^+ from the lumen of the phagosome to the cytoplasm, a region of the cell that is known to have a high concentration of the ion.[117] It is at these K^+ transport sites of the phagosome where ouabain, verapamil, reserpine and phenothiazines inhibit K^+ efflux; and thereby the concentration of K^+ within the phagosome would be maintained to levels needed for the acidification of the phagolysosome and activation of the hydrolases.[111,112] However, recent studies have shown that the killing activity of monocyte-derived macrophages, which are identical to those used in the experiments described in Figure 4, is dependent upon extracellular calcium and extracellular ATP,[118,119] both of which are under transport control mediated by Ca^{2+}-dependent ATPase. As is the case for the K^+ transport systems, the transport process for Ca^{2+} would be reversed in the phagosome if this system were to be retained in the plasma membrane that delimits the phagosome unit. Phagocytosis of the bacterium by macrophages should result in a phagosome that contains K^+ and Ca^{2+} transport

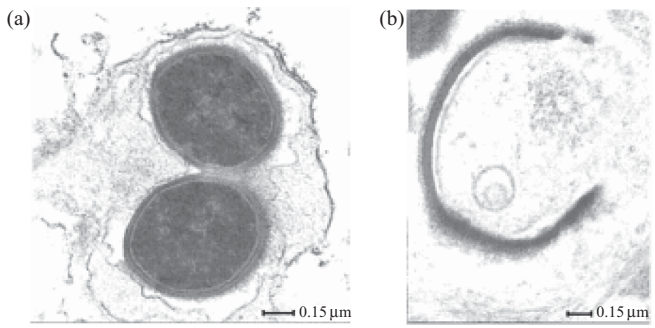

Figure 3. The *ex vivo* effects of thioridazine on the ultrastructure of *Staphylococcus aureus* phagocytosed by monocyte-derived macrophages. (a) Control; (b) thioridazine-treated 6 h post-phagocytosis; thioridazine (0.1 mg/L) was added to the medium 30 min after phagocytosis.[110]

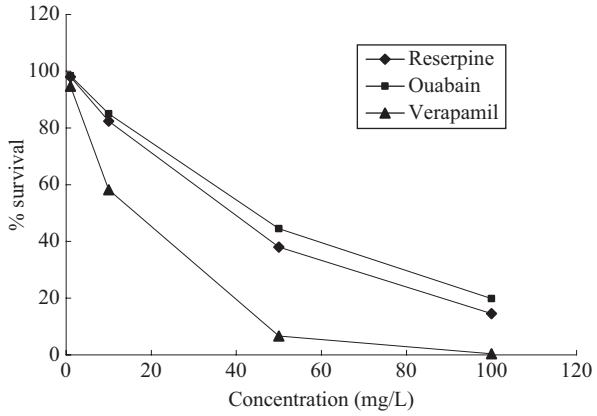

Figure 4. Effects of ouabain, verapamil and reserpine on the killing activity of human monocyte-derived macrophages.[114]

systems that pump out these ions to the cytoplasm of the cell. It is hypothesized that failure to kill bacteria present in the phagosome–lysosome is due to the low concentration of these ions in the complex that results from their extrusion to the cytoplasm. The inhibition of either one or both of these processes by a phenothiazine may establish conditions by which the concentration of these ions can be increased to a point where other ATP-driven membrane exchange systems involved in the cytosolic homeostasis are activated, leading to an intense hydrolytic activity of the phagosome–lysosome ATPases, in particular the V-ATPases.[113,118,119] The import of H^+ thereby creating the acidification of the lysosome component that had fused with the phagosome and the activation of the hydrolases needed for the killing of the bacterium. These ATP-driven membrane exchange systems must also be immune to ouabain, phenothiazines, etc, Figure 5 presents a hypothetical model which describes the aforementioned events that ultimately transform non-killer macrophages into effective killers. The phagocytosis of the bacterium (Figure 5a) results in a phagolysosome whose pumps extrude K^+ and Ca^{2+} from the vacuole to the cytoplasm (IV). When a phenothiazine is presented to the macrophage that has phagocytosed the mycobacterium (Figure 5b), the efflux of K^+ and Ca^{2+} from the phagolysosome is prevented, the build up of H^+ takes place, the hydrolases are now activated and the bacterium is digested (VII).

The search for inhibitors of efflux pumps that may also enhance the killing activity of non-killing macrophages

Our previous studies demonstrated unusual properties of an extract made from the Portuguese nuisance plant *Carpobrotus edulis*.[120,121] Among these were: (i) the ability to invoke Th1 responses of a variety of T-cell subsets as well as to stimulate the production of cytokines that are involved in the immune response to an infectious agent; (ii) the ability to render highly resistant mouse lymphoma cells that contain the MDR efflux pump transport pg1 completely susceptible to cytotoxic agents; (iii) the ability to inhibit the MDR efflux pump of these mouse lymphoma cells as evident from the increased retention of the efflux pump substrate rhodamine 1,2,3; and (iv) the ability to enhance the killing of intracellular (phagocytosed) *M. tuberculosis* as well as *Mycobacterium avium*,[122] whereas the highest concentration of the extract was devoid of any *in vitro* activity against these mycobacteria. Although it is not yet known whether the activities noted for the *C. edulis* extract are due to one or more substances [the extract and the inhibitors of K^+ transport (ouabain, reserpine and verapamil) enhance the killing of intracellular bacteria by non-killing macrophages whereas they do not have any *in vitro* activity against these bacteria], it seems possible that the activity noted for the extract is

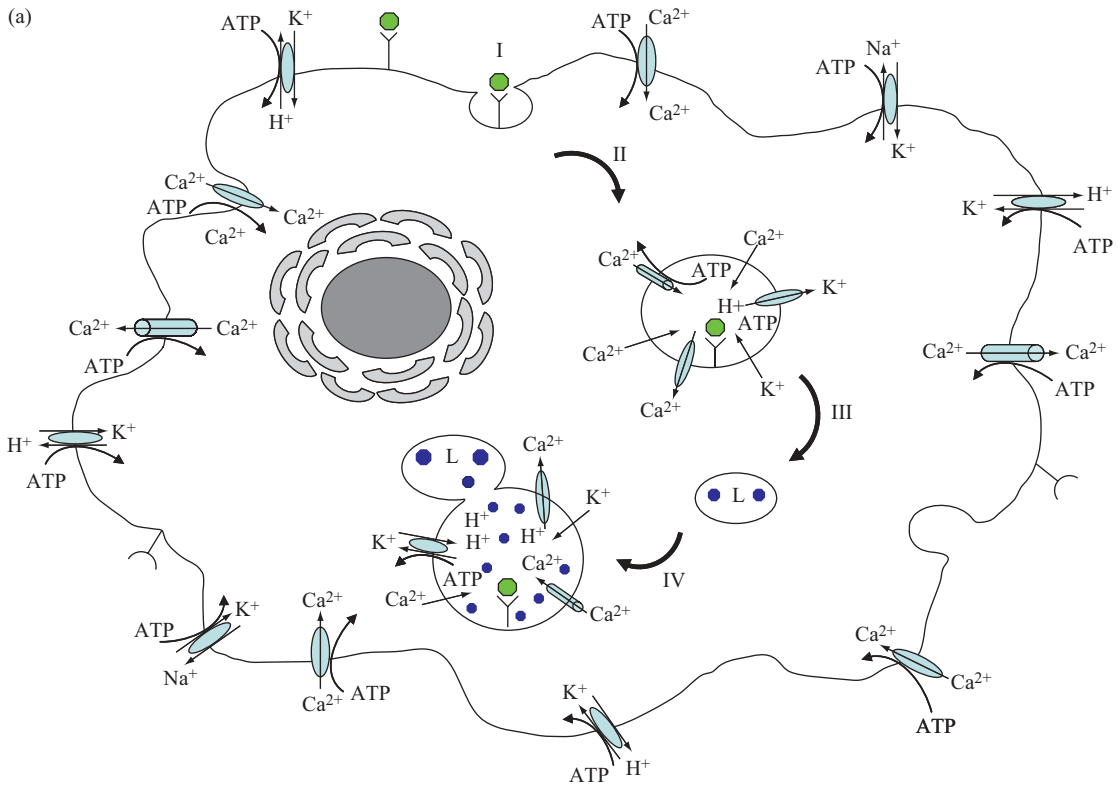

Figure 5. Hypothetical model suggested for the enhancement of killing of human monocyte-derived macrophages by phenothiazines and other efflux pumps. (a) Infected and untreated macrophage. (b) Infected and treated macrophage. Sequence of events described in the model: I, binding of bacteria to plasma membrane; II, invagination and formation of phagosome; III and IV, maturation of phagosome and fusion of the lysosome with the phagosome; V, binding of inhibitor of efflux pump and formation of vesicle; VI, fusion of vesicle containing the inhibitor of efflux pump with matured phagolysosome; VII, inhibition of Ca^{2+} and K^+ efflux by inhibitor of efflux pump; K^+ leaks into phagolysosome with cytosolic homeostasis mechanisms activated leading to an increased activity of the ATPases; acidification of the phagolysosome; activation of hydrolases; killing of bacteria. Note: hydrolysis of ATP to ADP by ATPases is not shown in the diagram.

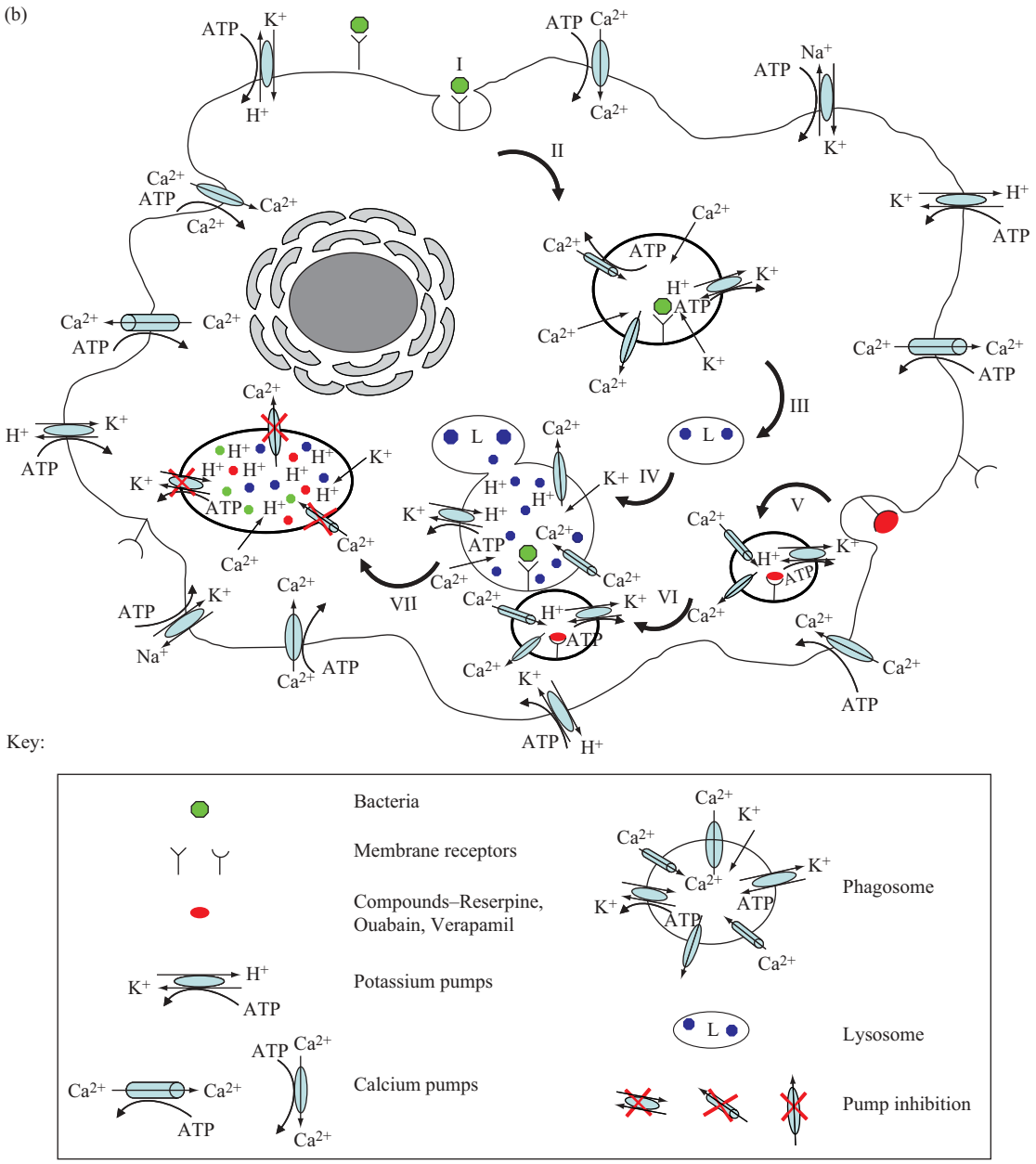

Figure 5. Continued.

due to one agent, and that this agent manifests its effects via the inhibition of K^+ transport. Other plant extracts as well as plant-derived compounds that have been shown to inhibit efflux pumps of cancer cells also have activity against phagocytosed bacteria. The suggestion that inhibitors of the P-glycoprotein of mammalian cells may also inhibit the efflux pump of bacteria receives support from the studies that used piperidine, an alkaloid isolated from the fruits of *Piper longum*[123] and its derivative piperine, to inhibit the P-glycoprotein of Caco-2 cells,[124] as well to increase the retention of ethidium bromide in *S. aureus* by the inhibition of efflux activity.[125] Polyphenols obtained from plant sources have been shown to inhibit efflux pumps of Caco-2 cells[126] and have been shown to enhance the killing of intracellular *M. tuberculosis*.[127]

Other plant-derived agents that have activity against efflux pumps of cancer cells may also enhance the killing activity of macrophages against bacteria, perhaps even mycobacteria. Voacamine, a bisindolic alkaloid from *Peschiera fuchsiaefolia*, induces a significant increase of drug retention in cancer cells by its ability to inhibit the MDR transporter protein, P-glycoprotein.[128] Irofulven, a novel anticancer agent derived from the mushroom, reverses the resistance of cancer cells to cytotoxic agents by inhibiting the MDR efflux pump responsible for this resistance.[129] Curcumin mixture and three major curcuminoids purified from turmeric (curcumin I, II and III) have been shown to modulate the function of MDR protein 1 (MRP1) of HEK293 cells stably transfected with MRP1-pcDNA3.1.[130] These and many other plant-derived compounds that are active

against efflux pumps of cancer cells may serve as lead compounds for the synthesis of new agents that have activity against intracellular bacteria.

Concluding remarks

MDR-TB is an intracellular infection of the non-killing macrophage of the lung, so any drug that is to be effective must have activity at this site. Conventionally, anti-tubercular drugs are designed to have direct activity against intracellular MDR-TB and, as has been the case for all other antibiotics, resistance ensues with usage. Because the non-killer macrophage can be transformed into an effective killer by a variety of compounds that inhibit K^+ transport, perhaps it would be wiser to develop drugs that enhance the killing activity of these cells inasmuch as this approach would not be subject to any resistance as is inevitably the case for conventional antibiotics.

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Transparency declarations

None to declare.

References

1. Gernaey AM, Minnikin DE, Copley MS *et al*. Mycolic acids and ancient DNA confirm an osteological diagnosis of tuberculosis. *Tuberculosis* 2001; **81**: 259–65.

2. Morell V. Mummy settles TB antiquity debate. *Science* 1994; **263**: 1686–7.

3. Stender HS, Eckel H. Pulmonary tuberculosis today-revisited. *Radiologe* 1981; **21**: 116–21.

4. Hinman AR, Judd JM, Kolnik JP *et al*. Changing risks in tuberculosis. *Am J Epidemiol* 1976; **103**: 486–97.

5. Amaral L, Kristiansen JE, Viveiros M *et al*. Activity of phenothiazines against antibiotic-resistant *Mycobacterium tuberculosis*: a review supporting further studies that may elucidate the potential use of thioridazine as anti-tuberculosis therapy. *J Antimicrob Chemother* 2001; **47**: 505–11.

6. Trends in tuberculosis—United States 1998–2003. Centers for Disease Control and Prevention (CDC). *MMWR Morb Mortal Wkly Rep* 2004; **53**: 209–14.

7. Roca C, Balanzo X, Fernandez-Roure JL *et al*. Imported diseases in African immigrants in Spain: study of 1321 patients. *Med Clin* (Barc) 2002; **119**: 616–19.

8. Raviglione MC, Sudre P, Rieder HL *et al*. Secular trends of tuberculosis in western Europe. *Bull World Health Org* 1993; **71**: 297–306.

9. McKenna MT, McCray E, Jones JL *et al*. The fall after the rise: Tuberculosis in the United States, 1991 through 1994. *Am J Public Health* 1998; **88**: 1059–63.

10. Kamholz SL. Resurgence of tuberculosis: the perspective a dozen years later. *J Assoc Acad Minor Phys* 1996; **7**: 83–6.

11. Munsiff SS, Nivin B, Sacajiu G *et al*. Persistence of a highly resistant strain of tuberculosis in New York City during 1990–1999. *J Infect Dis* 2003; **188**: 356–63.

12. Munsiff SS, Bassoff T, Nivin B *et al*. Molecular epidemiology of multidrug-resistant tuberculosis, New York City, 1995–1997. *Emerg Infect Dis* 2002; **8**: 1230–8.

13. Rao GG. Risk factors for the spread of antibiotic-resistant bacteria. *Drugs* 1998; **55**: 323–30.

14. Amaral L, Viveiros M, Molnar J. Antimicrobial activity of phenothiazines. *In Vivo* 2004; **18**: 725–31.

15. Drobniewski FA, Balabanova YM. The diagnosis and management of multiple-drug-resistant-tuberculosis at the beginning of the new millennium. *Int J Infect Dis* 2002; **6** Suppl 1: 21–31.

16. World Health Organization 2005. *Global Tuberculosis Control—Surveillance, Planning, Financing*. WHO Report 2005, 247 pp. World Health Organization, Geneva, Switzerland.

17. Atasever A, Bacakoglu F, Toz H *et al*. Tuberculosis in renal transplant recipients on various immunosuppressive regimens. *Nephrol Dial Transplant* 2005; **20**: 797–802.

18. Gomez-Reino JJ, Carmona L, Valverde VR *et al*. Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. *Arthritis Rheum* 2003; **48**: 2122–7.

19. van Pinxteren LA, Cassidy JP, Smedegaard BH *et al*. Control of latent *Mycobacterium tuberculosis* infection is dependent on CD8 T cells. *Eur J Immunol* 2000; **30**: 3689–98.

20. Trautmann M, Ruhnke M, Held T *et al*. Complicated tuberculosis and residual disease. *Immunobiology* 1994; **191**: 344–50.

21. Ibrahim EM, Uwaydah A, al-Mulhim FA *et al*. Tuberculosis in patients with malignant disease. *Indian J Cancer* 1989; **26**: 53–7.

22. Duzhyi ID, Blyzniuk MD, Boiko VI *et al*. Tuberculous pleuritis and pregnancy. *Lik Sprava* 2002 Jul–Sep;(5–6): 69–71.

23. Vo QT, Stettler W, Crowley K. Pulmonary tuberculosis in pregnancy. *Prim Care Update Ob Gyn* 2000; **7**: 244–9.

24. Ordway DJ, Costa L, Martins M *et al*. Increased Interleukin-4 production by CD8 and gamma delta T cells in health-care workers is associated with the subsequent development of active tuberculosis. *J Infect Dis* 2004; **190**: 756–66.

25. Amaral L, Viveiros M, Kristiansen JE. Phenothiazines: potential alternatives for the management of antibiotic resistant infections of tuberculosis and malaria in developing countries. *Trop Med Int Health* 2001; **6**: 1016–22.

26. Kristiansen JE, Amaral L. The potential management of resistant infections with non-antibiotics. *J Antimicrob Chemother* 1997; **40**: 319–27.

27. Zhang Y. The Magic Bullets and tuberculosis drug targets. *Ann Rev Pharm Toxicol* 2005; **45**: 529–64.

28. Amaral L, Kristiansen JE. Phenothiazines: an alternative to conventional therapy for the initial management of suspected multidrug resistant tuberculosis. A call for studies. *Int J Antimicrob Agents* 2000; **14**: 173–6.

29. Amaral L, Lorian V. Enzymatic conversion of clindamycin by the human neutrophil. In: Gullinsen G, ed. *The Influence of Antibiotics on the Host–Parasite Relationship*. Springer-Verlag, Berlin-New York, 1988; 102–7.

30. Wainwright M, Amaral L. The phenothiazinium chromophore and the evolution of antimalarial drugs. *Trop Med Int Health* 2005; **10**: 501–11.

31. Wolfart K, Molnar A, Kawase M *et al*. Effects of trifluoromethyl ketones on the motility of *Proteus vulgaris*. *Biol Pharm Bull* 2004; **27**: 1462–4.

32. Kristiansen JE. The antimicrobial activity of psychotherapeutic drugs and stereo-isomeric analogues. *Dan Med Bull* 1990; **37**: 165–82.

33. Kristiansen JE, Mortensen I. Antibacterial effect of four phenothiazines. *Pharmacol Toxicol* 1987; **60**: 100–3.

34. Amaral L, Kristiansen J, Lorian V. Synergic effect of chlorpromazine on the activity of some antibiotics. *J Antimicrob Chemother* 1992; **30**: 556–8.

35. Amaral L, Lorian V. Effects of chlorpromazine on the cell envelope proteins of *Escherichia coli. Antimicrob Agents Chemother* 1991; **35**: 1923–4.

36. Bodoni P. Dell' azione sedative del bleu di metilene in vaire frome di psicosi. *Clinica Medica Italiana* 1899; **2**: 445–51.

37. Amaral L, Kristiansen JE. Phenothiazines: potential management of Creutzfeldt–Jacob disease and its variants. *Int J Antimicrob Agents* 2002; **20**: 305–6.

38. Williams JD. The Garrod Lecture. Selective toxicity and concordant pharmacodynamics of antibiotics and other drugs. *J Antimicrob Chemother* 1995; **35**: 721–37.

39. Zhang Y, Post-Martens K, Denkin S. New drug candidates and therapeutic targets for tuberculosis therapy. *Drug Discov Today* 2006; **11**: 21–7.

40. Horne NW. Drug-resistant tuberculosis: a review of the world situation. *Tubercle* 1969; **50** Suppl: 2–12.

41. Jancik E. The treatment of tuberculous-patients with resistant tubercule bacilli. *Wien Med Wochenschr* 1966; **116**: 925–9.

42. Steiner M. Newer and second-line drugs in the treatment of drug-resistant tuberculosis in children. *Med Clin North Am* 1967; **51**: 1153–67.

43. Manten A. The non-medical use of antibiotics and the risk of causing microbial drug-resistance. *Bull World Health Org* 1963; **29**: 387–400.

44. von Wasielewski E. On the problem of bacterial persistency. *Internist* (Berl) 1967; **8**: 204–8.

45. Zirakzadeh A, Patel R. Vancomycin-resistant enterococci: colonization, infection, detection, and treatment. *Mayo Clin Proc* 2006; **81**: 529–36.

46. Enia F, Bella R, Mineo R *et al*. An alarming problem in the therapy of infective endocarditis: the development of antibiotic-resistant strains. *Ital Heart J Suppl* 2005; **6**: 121–7.

47. Andersson DI. Persistence of antibiotic resistant bacteria. *Curr Opin Microbiol* 2003; **6**: 452–6.

48. Gerrish PJ, Garcia-Lerma JG. Mutation rate and the efficacy of antimicrobial drug treatment. *Lancet Infect Dis* 2003; **3**: 28–32.

49. World Health Organization. The WHO/IUTALD Global Project on Anti-Tuberculosis Drug Resistance Surveillance 1994–1997, World Health Organization, Geneva, Switzerland, 1998; 1–227.

50. Crowle AJ, Douvas GS, May MH. Chlorpromazine: a drug potentially useful for treating mycobacterial infections. *Chemotherapy* 1992; **38**: 410–19.

51. Molnar J, Beladi I, Foldes I. Studies on antituberculotic action of some phenothiazine derivatives *in vitro. Zentralbl Bakteriol [Orig A]* 1977; **239**: 521–6.

52. Kristiansen JE, Vergmann B. The antibacterial effect of selected phenothiazines and thioxanthenes on slow-growing mycobacteria. *Acta Pathol Microbiol Immunol Scand [B]* 1986; **94**: 393–8.

53. Amaral L, Kristiansen JE, Abebe LS *et al*. Inhibition of the respiration of multi-drug resistant clinical isolates of *Mycobacterium tuberculosis* by thioridazine: potential use for initial therapy of freshly diagnosed tuberculosis. *J Antimicrob Chemother* 1996; **38**: 1049–53.

54. Bettencourt MV, Bosne-David S, Amaral L. Comparative *in vitro* activity of phenothiazines against multidrug-resistant *Mycobacterium tuberculosis*. *Int J Antimicrob Agents* 2000; **16**: 69–71.

55. Viveiros M, Amaral L. Enhancement of antibiotic activity against poly-drug resistant *Mycobacterium tuberculosis* by phenothiazines. *Int J Antimicrob Agents* 2001; **17**: 225–8.

56. Gadre DV, Talwar V. *In vitro* susceptibility testing of *Mycobacterium tuberculosis* strains to trifluoperazine. *J Chemother* 1999; **11**: 203–6.

57. Gadre DV, Talwar V, Gupta HC *et al*. Effect of trifluoperazine, a potential drug for tuberculosis with psychotic disorders, on the growth of clinical isolates of drug resistant *Mycobacterium tuberculosis*. *Int Clin Psychopharmacol* 1998; **13**: 129–31.

58. Reddy MV, Nadadhur G, Gangadharam PR. *In-vitro* and intracellular antimycobacterial activity of trifluoperazine. *J Antimicrob Chemother* 1996; **37**: 196–7.

59. Ratnakar P, Rao SP, Sriramarao P *et al*. Structure–antitubercular activity relationship of phenothiazine-type calmodulin antagonists. *Int Clin Psychopharmacol* 1995; **10**: 39–43.

60. Ratnakar P, Murthy PS. Trifluoperazine inhibits the incorporation of labelled precursors into lipids, proteins and DNA of *Mycobacterium tuberculosis* H37Rv. *FEMS Microbiol Lett* 1993; **110**: 291–4.

61. Chakrabarty AN, Bhattacharya CP, Dastidar SG. Antimycobacterial activity of methdilazine (Md), an antimicrobic phenothiazine. *APMIS* 1993; **101**: 449–54.

62. Ratnakar P, Murthy PS. Antitubercular activity of trifluoperazine, a calmodulin antagonist. *FEMS Microbiol Lett* 1992; **76**: 73–6.

63. Roberts KR, Hammersley PA. *In vitro* uptake of gallium and chlorpromazine by mouse tumour cells. *Eur J Nucl Med* 1985; **10**: 366–8.

64. Kuratomi Y, Akiyama S, Ono M *et al*. Thioridazine enhances lysosomal accumulation of epidermal growth factor and toxicity of conjugates of epidermal growth factor with Pseudomonas exotoxin. *Exp Cell Res* 1986; **162**: 436–48.

65. Tapper H, Sundler R. Role of lysosomal and cytosolic pH in the regulation of macrophage lysosomal enzyme secretion. *Biochem J* 1990; **272**: 407–14.

66. Ishizaki J, Yokogawa K, Hirano M *et al*. Contribution of lysosomes to the subcellular distribution of basic drugs in the rat liver. *Pharm Res* 1996; **13**: 902–6.

67. Daniel WA, Wojcikowski J. Contribution of lysosomal trapping to the total tissue uptake of psychotropic drugs. *Pharmacol Toxicol* 1997; **80**: 62–8.

68. Daniel WA, Wojcikowski J. Interactions between promazine and antidepressants at the level of cellular distribution. *Pharmacol Toxicol* 1997; **81**: 259–64.

69. Daniel WA, Wojcikowski J. The role of lysosomes in the cellular distribution of thioridazine and potential drug interactions. *Toxicol Appl Pharmacol* 1999; **158**: 115–24.

70. Daniel WA, Wojcikowski J. Lysosomal trapping as an important mechanism involved in the cellular distribution of perazine and in pharmacokinetic interaction with antidepressants. *Eur Neuropsychopharmacol* 1999; **9**: 483–91.

71. Daniel WA, Wojcikowski J, Palucha A. Intracellular distribution of psychotropic drugs in the grey and white matter of the brain: the role of lysosomal trapping. *Br J Pharmacol* 2001; **134**: 807–14.

72. Wojcikowski J, Daniel WA. Thioridazine-fluoxetine interaction at the level of the distribution process *in vivo*. *Pol J Pharmacol* 2002; **54**: 647–54.

73. Ordway D, Viveiros M, Leandro C *et al*. Clinical concentrations of thioridazine kill intracellular multidrug-resistant *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2003; **47**: 917–22.

74. Ordway D, Viveiros M, Leandro C *et al*. Chlorpromazine has intracellular killing activity against phagocytosed *Staphylococcus aureus* at clinical concentrations. *J Infect Chemother* 2002; **8**: 227–31.

75. Ordway D, Viveiros M, Leandro C *et al*. Intracellular activity of clinical concentrations of phenothiazines including thioridiazine against phagocytosed *Staphylococcus aureus*. *Int J Antimicrob Agents* 2002; **20**: 34–43.

76. Yano T, Li LS, Weinstein E *et al*. Steady-state kinetics and inhibitory action of antitubercular phenothiazines on *Mycobacterium tuberculosis* type-II NADH-menaquinone oxidoreductase (NDH-2). *J Biol Chem* 2006; **281**: 11 456–63.

77. Weinstein EA, Yano T, Li LS *et al*. Inhibitors of type II NADH:menaquinone oxidoreductase represent a class of antitubercular drugs. *Proc Natl Acad Sci USA* 2005; **102**: 4548–53.

78. Sun H, Liu X, Xiong Q *et al*. Chronic inhibition of cardiac Kir2.1 and HERG potassium channels by celastrol with dual effects on both ion conductivity and protein trafficking. *J Biol Chem* 2006 Mar; **281**: 5877–84.

79. Kim KS, Kim EJ. The phenothiazine drugs inhibit hERG potassium channels. *Drug Chem Toxicol* 2005; **28**: 303–13.

80. Plishker GA. Phenothiazine inhibition of calmodulin stimulates calcium-dependent potassium efflux in human red blood cells. *Cell Calcium* 1984; **5**: 177–85.

81. Tolon R, Franco FS, Villuendas G *et al*. Potassium depolarization-induced cAMP stimulates somatostatin mRNA levels in cultured diencephalic neurons. *Brain Res* 2000; **868**: 338–46.

82. Chukhlova EA, Sadykov IuKh, Kholmukhamedov EL *et al*. Effect of trimecaine, ajmaline, stenopril and chloracizine on fluctuations in K+ currents in rat liver mitochondria. *Ukr Biokhim Zh* 1984; **56**: 207–10.

83. Weiss B, Prozialeck W, Cimino M *et al*. Pharmacological regulation of calmodulin. *Ann N Y Acad Sci* 1980; **356**: 319–45.

84. Sarkisova S, Patrauchan MA, Berglund D *et al*. Calcium-induced virulence factors associated with the extracellular matrix of mucoid *Pseudomonas aeruginosa* biofilms. *J Bacteriol* 2005; **187**: 4327–37.

85. Yonekawa T, Ohnishi Y, Horinouchi S. A calmodulin-like protein in the bacterial genus Streptomyces. *FEMS Microbiol Lett* 2005; **244**: 315–21.

86. Reddy PT, Prasad CR, Reddy PH *et al*. Cloning and expression of the gene for a novel protein from *Mycobacterium smegmatis* with functional similarity to eukaryotic calmodulin. *Bacteriol* 2003; **185**: 5263–8.

87. Rigden DJ, Jedrzejas MJ, Galperin MY. An extracellular calcium-binding domain in bacteria with a distant relationship to EF-hands. *FEMS Microbiol Lett* 2003; **221**: 103–10.

88. Dhople AM. *In vitro* activities of phenothiazine-type calmodulin antagonists against *Mycobacterium leprae*. *Microbios* 1999; **98**: 113–21.

89. Sarma PV, Sarma PU, Murthy PS. Isolation, purification and characterization of intracellular calmodulin like protein (CALP) from *Mycobacterium phlei*. *FEMS Microbiol Lett* 1998; **159**: 27–34.

90. Nagai M, Watanabe M, Endoh M *et al*. Comparison of characterization among *Bordetella* calmodulin-like protein, bovine brain calmodulin and *Escherichia coli* acyl-carrier protein. *Biol Pharm Bull* 1997; **20**: 1036–8.

91. Nagai M, Endoh M, Danbara H *et al*. Purification and characterization of *Bordetella* calmodulin-like protein. *FEMS Microbiol Lett* 1994; **116**: 169–74.

92. Salih FA, Kaushik NK, Sharma P *et al*. Calmodulin-like activity in mycobacteria. *Indian J Biochem Biophys* 1991; **28**: 491–5.

93. Fry IJ, Villa L, Kuehn GD *et al*. Calmodulin-like protein from *Bacillus subtilis*. *Biochem Biophys Res Commun* 1986; **134**: 212–7.

94. Kaatz GW, Moudgal VV, Seo SM *et al*. Phenothiazines and thioxanthenes inhibit multidrug efflux pump activity in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2003; **47**: 719–26.

95. Nacsa J, Nagy L, Sharples D *et al*. The inhibition of SOS-responses and MDR by phenothiazine-metal complexes. *Anticancer Res* 1998; **18**: 3093–8.

96. Molnar J, Hever A, Fakla I *et al*. Inhibition of the transport function of membrane proteins by some substituted phenothiazines in *E. coli* and multidrug resistant tumor cells. *Anticancer Res* 1997; **17**: 481–6.

97. Viveiros M, Jesus A, Brito M *et al*. Inducement and reversal of tetracycline resistance in *Escherichia coli* K-12 and expression of proton gradient-dependent multidrug efflux pump genes. *Antimicrob Agents Chemother* 2005; **49**: 3578–82.

98. Viveiros M, Leandro C, Amaral L. Mycobacterial efflux pumps and chemotherapeutic implications. *Int J Antimicrob Agents* 2003; **22**: 274–8.

99. Viveiros M, Portugal I, Bettencourt R *et al*. Isoniazid-induced transient high-level resistance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2002; **46**: 2804–10.

100. Kristiansen MM, Leandro C, Ordway D *et al*. Phenothiazines alter resistance of methicillin-resistant strains of *Staphylococcus aureus* (MRSA) to oxacillin *in vitro*. *Int J Antimicrob Agents* 2003; **22**: 250–3.

101. Kristiansen MM, Leandro C, Ordway D *et al*. Thioridazine reduces resistance of methicillin-resistant *Staphylococcus aureus* by inhibiting a reserpine-sensitive efflux pump. *In Vivo* 2006; **20**: 361–6.

102. Piddock LJ. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clin Microbiol Rev* 2006; **19**: 382–402.

103. Hoffmann U, Kroemer HK. The ABC transporters MDR1 and MRP2: multiple functions in disposition of xenobiotics and drug resistance. *Drug Metab Rev* 2004; **36**: 669–701.

104. Sinha R, Islam MM, Bhadra K *et al*. The binding of DNA intercalating and non-intercalating compounds to A-form and protonated form of poly(rC).poly(rG): spectroscopic and viscometric study. *Bioorg Med Chem* 2006; **14**: 800–14.

105. Rohs R, Sklenar H. Methylene blue binding to DNA with alternating AT base sequence: minor groove binding is favored over intercalation. *J Biomol Struct Dyn* 2004; **21**: 699–711.

106. de Mol NJ, Posthuma RM, Mohn GR. Induction of repairable DNA damage in *Escherichia coli* and interaction with DNA *in vitro* by the radical cation of chlorpromazine. *Chem Biol Interact* 1983; **47**: 223–37.

107. Webb RB, Hass BS. Biological effects of dyes on bacteria. VI. Mutation induction by acridine orange and methylene blue in the dark with special reference to *Escherichia coli* WP6 (polA1). *Mutat Res* 1984; **137**: 1–6.

108. Webb RB, Hass BS, Kubitschek HE. Photodynamic effects of dyes on bacteria. II. Genetic effects of broad-spectrum visible light in the presence of acridine dyes and methylene blue in chemostat cultures of *Escherichia coli*. *Mutat Res* 1979; **59**: 1–13.

109. Boshoff HI, Myers TG, Copp BR *et al*. The transcriptional responses of *Mycobacterium tuberculosis* to inhibitors of metabolism: novel insights into drug mechanisms of action. *J Biol Chem* 2004; **279**: 40 174–84.

110. Martins M, Bleiss W, Marko A *et al*. Clinical concentrations of thioridazine enhance the killing of intracellular methicillin-resistant *Staphylococcus aureus*: an *in vivo*, *ex vivo* and electron microscopy study. *In Vivo* 2004; **18**: 787–94.

111. Reeves EP, Lu H, Jacobs HL *et al*. Killing activity of neutrophils is mediated through activation of proteases by K+ flux. *Nature* 2002; **416**: 291–7.

112. Ahluwalia J, Tinker A, Clapp LH *et al*. The large-conductance Ca^{2+}–activated K^+ channel is essential for innate immunity. *Nature* 2004; **427**: 853–8.

113. Pillay CS, Elliott E, Dennison C. Endolysosomal proteolysis and its regulation. *Biochem J* 2002; **363**: 417–29.

114. Martins M, Viveiros M, Ordway D *et al*. Reserpine, ouabain and the calcium channel blocker verapamil, cause intracellular killing of *Staphylococcus aureus*. *Res J Microbiol* 2006; **1**: 203–9.

115. Aydemir-Koksoy A, Allen JC. Regulation of Na(+) pump expression by vascular smooth muscle cells. *Am J Physiol Heart Circ Physiol* 2001; **280**: H1869–74.

116. Terreros DA, Tiedemann K. Renal ontogeny: epithelial transport in the mammalian mesonephric proximal tubule. *Ann Clin Lab Sci* 1991; **21**: 187–96.

117. Gribble FM, Loussouarn G, Tucker SJ *et al*. A novel method for measurement of submembrane ATP concentration. *J Biol Chem* 2000; **275**: 30 046–9.

118. Kusner DJ, Barton JA. ATP stimulates human macrophages to kill intracellular virulent *Mycobacterium tuberculosis* via calcium-dependent phagosome–lysosome fusion. *J Immunol* 2001; **167**: 3308–15.

119. Fairbairn IP, Stober CB, Kumararatne DS. ATP-mediated killing of intracellular mycobacteria by macrophages is a P2X7-dependent process inducing bacterial death by phagosome–lysosome fusion. *J Immunol* 2001; **167**: 3300–07.

120. Ordway D, Hohmann J, Viveiros M *et al Carpobrotus edulis* methanol extract inhibits the MDR efflux pumps, enhances killing of phagocytosed *S. aureus* and promotes immune modulation. *Phytother Res* 2003; **17**: 512–19.

121. Martins M, Ordway D, Kristiansen M *et al*. Inhibition of the *Carpobrotus edulis* methanol extract on the growth of phagocytosed multidrug-resistant *Mycobacterium* tuberculosis and methicillin-resistant *Staphylococcus aureus*. *Fitoterapia* 2005; **76**: 96–9.

122. Viveiros M, Martins M, Couto I *et al*. The *in vitro* activity of phenothiazines against *Mycobacterium avium*: potential of thioridazine for therapy of the co-infected AIDS patient. *In Vivo* 2005; **19**: 733–6.

123. Lee SA, Hong SS, Han XH *et al*. Piperine from the fruits of *Piper longum* with inhibitory effect on monoamine oxidase and antidepressant-like activity. *Chem Pharm Bull* 2005; **53**: 832–5.

124. Bhardwaj RK, Glaeser H, Becquemont L *et al*. Piperine, a major constituent of black pepper, inhibits human P-glycoprotein and CYP3A4. *J Pharmacol Exp Ther* 2002; **302**: 645–50.

125. Khan IA, Mirza ZM, Kumar A *et al*. Piperine, a phytochemical potentiator of ciprofloxacin against *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2006; **50**: 810–12.

126. Sergent T, Garsou S, Schaut A *et al*. Differential modulation of ochratoxin A absorption across Caco-2 cells by dietary polyphenols, used at realistic intestinal concentrations. *Toxicol Lett* 2005; **159**: 60–70.

127. Anand PK, Kaul D, Sharma M. Green tea polyphenol inhibits *Mycobacterium tuberculosis* survival within human macrophages. *Int J Biochem Cell Biol* 2006; **38**: 600–9.

128. Meschini S, Marra M, Condello M *et al*. Voacamine, an alkaloid extracted from *Peschiera fuchsiaefolia*, inhibits P-glycoprotein action in multidrug-resistant tumor cells. *Int J Oncol* 2005; **27**: 1597–603.

129. Poindessous V, Koeppel F, Raymond E *et al*. Marked activity of irofulven toward human carcinoma cells: comparison with cisplatin and ecteinascidin. *Clin Cancer Res* 2003; **9**: 2817–25.

130. Chearwae W, Wu CP, Chu HY *et al*. Curcuminoids purified from turmeric powder modulate the function of human multidrug resistance protein 1 (ABCC1). *Cancer Chemother Pharmacol* 2006; **57**: 376–88.