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The *In Vitro* Activity of Phenothiazines Against *Mycobacterium avium*: Potential of Thioridazine for Therapy of the Co-infected AIDS Patient

MIGUEL VIVEIROS[1], MARTA MARTINS[1], ISABEL COUTO[1], JETTE E. KRISTIANSEN[2], JOSEPH MOLNAR[3] and LEONARD AMARAL[1]

[1]*Unit of Mycobacteriology, UPMM, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Rua da Junqueira 96, 1349-019 Lisboa, Portugal;*
[2]*Department of Clinical Microbiology, Sonderborg Sygehus, South Danish University, 6400 Sonderborg, Denmark;*
[3]*Institute of Medical Microbiology, Albert-Szent Gyorgyi School of Medicine, Dom tér 10/11, 6720 Szeged, Hungary*

Abstract. *Patients presenting with Acquired Immune Deficiency Syndrome (AIDS) are predisposed to co-infection with Mycobacterium avium. The management of such patients is problematic due to underlying immuno-incompetence and the high resistance of M. avium to most non-toxic compounds. Therefore, the need for effective agents is obvious. Because phenothiazines, especially the relatively mild thioridazine, have significant activity against Mycobacterium tuberculosis, we investigated the in vitro activity of chlorpromazine, thioridazine, promazine, promethazine and desipramine against a reference and clinical strains of M. avium. The results obtained show that whereas all of the phenothiazines employed in this study had an minimum inhibitory concentration (MIC) against the strains studied that ranged from ca. 10 to >50 mg/L, as was previously shown for M. tuberculosis, thioridazine was the most active of the group against M. avium.*

Phenothiazines have been shown to have *in vitro* activity against sensitive and drug-resistant strains of *Mycobacterium tuberculosis* (1-4). Nevertheless, the concentrations of these agents necessary to inhibit the growth of these bacteria is well beyond that clinically possible (5-8). However, chlorpromazine has activity against intracellular antibiotic-susceptible (9, 10) and antibiotic-resistant intracellular strains of *M. tuberculosis* (10) at concentrations in the medium which are similar (9) or below (10) those that are clinically employed. Because chlorpromazine causes frequent and serious side-effects when administered chronically (11), it is not a drug of choice for the management of multidrug-resistant (MDR-TB) infections. However, since the milder phenothiazine, thioridazine, is as effective *in vitro* (3, 4) and *ex vivo* (phagocytosed) MDR-TB (10) as is chlorpromazine (10), the use of thioridazine for the management of MDR-TB, where all other derivatives have failed, has been suggested (7, 12).

Chlorpromazine was shown by Crowle *et al.*, in 1992, to have activity against *M. avium* that had been phagocytosed by human macrophages at concentrations in the medium which were similar to those employed for the therapy of psychosis (9). Based upon this evidence, we studied the *in vitro* activity of chlorpromazine and four other phenothiazines conventionally employed for the management of psychosis against strains of *M. avium*.

Materials and Methods

Materials. Chlorpromazine (CPZ), thioridazine (TZ), promazine (PMZ), promethazine (PMTZ) and desipramine (DSP) were purchased from Sigma Aldrich Quımica SA, Madrid, Spain. Stock solutions of each phenothiazine were freshly prepared, as previously described (4).

Bacteria. The strains of *Mycobacterium avium* employed in this study were the *M. avium* CIP 14 031 002-S4 (Pasteur Institute, Paris, France), which has been maintained in our laboratory since 1996, and a clinical isolate obtained from a patient presenting with AIDS. Isolation of *M. avium* was done in BACTEC MGIT 960 tubes (Middlebrook 7H9 broth) (Becton-Dickinson Diagnostic Instrument Systems, Towson, MD, USA), and identification as the *M. avium* complex was conducted by the Accuprobe hybridisation probes (Gen-Probe Inc., San Diego, CA, USA), methods routinely

Correspondence to: Leonard Amaral, Unit of Mycobacteriology, UPMM, Institute of Hygiene and Tropical Medicine, Universidade Nova de Lisboa, Rua da Junqueira 96, 1349-019 Lisboa, Portugal. Tel: 351 21 365 2653, Fax: 351 21 363 2105, e-mail: lamaral@ihmt.unl.pt

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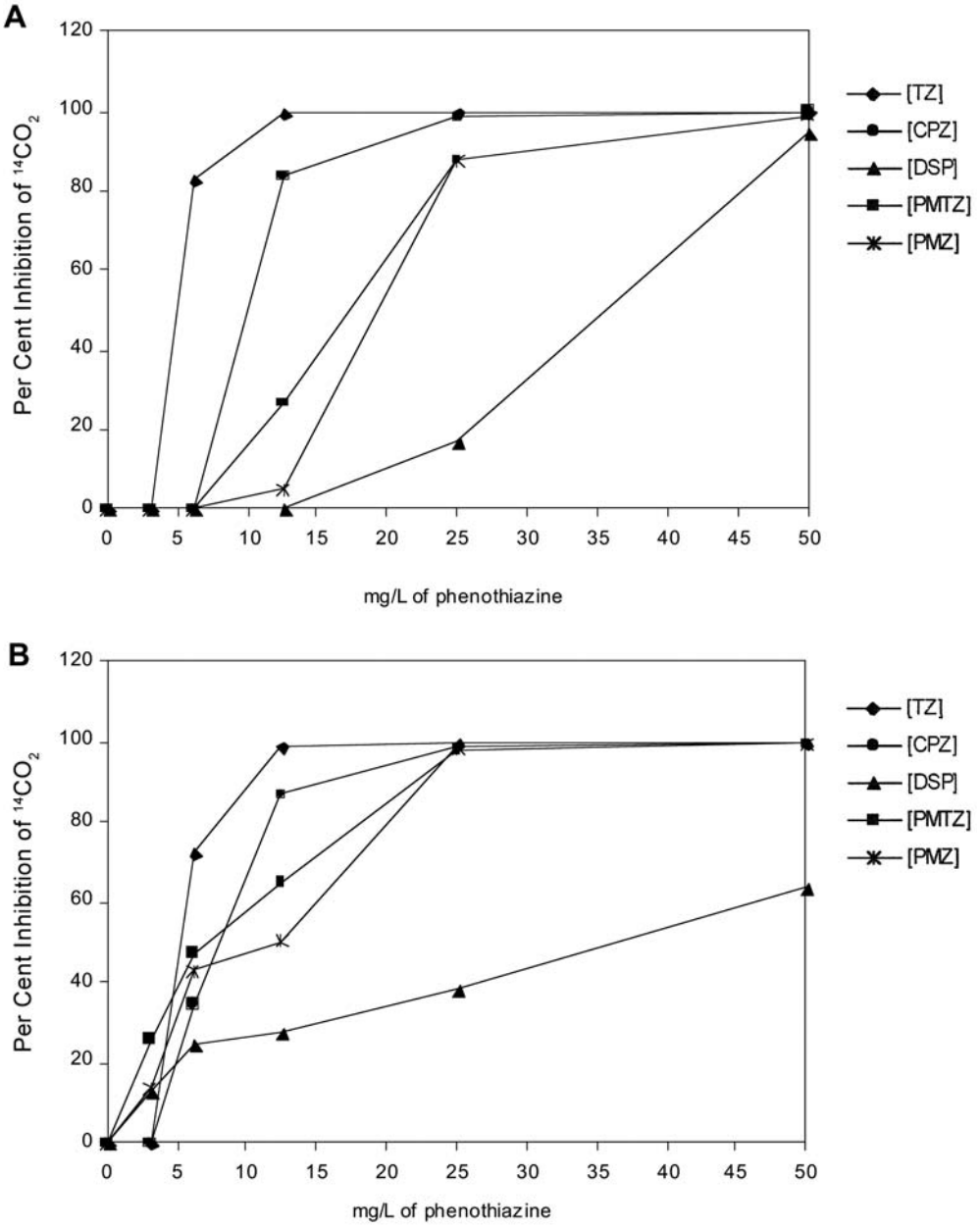

Figure 1. *The in vitro activity of chlorpromazine (CPZ), thioridazine (TZ), promazine (PMZ), promethazine (PMTZ) and desipramine (DSP) against Mycobacterium avium. Mycobacterium avium strain CIP 14 031 002-S4 (Pasteur Institute, Paris, France) (A) and a Mycobacterium avium strain isolated from an AIDS patient (B) were incubated in Bactec 12B vials that contained concentrations of each phenothiazine that ranged from 0.0 to 50 mg/L. The experiment was repeated twice and essentially the same quantitative data was obtained. The results represent the data obtained from one such experiment and describe the activity against both strains.*

employed in the Mycobacteriology Laboratory of the Institute of Hygiene and Tropical Medicine of Lisbon, Portugal (13).

Method for assessment of the in vitro effects of the phenothiazines against M. avium. M. avium strains were cultured in Middlebrook 7H9 broth, Bactec 12B vials (Becton-Dickinson Diagnostic Instrument Systems), until maximum growth was achieved. From these cultures, aliquots were transferred to vials and the optical density adjusted to a 0.5 McFarland standard. These vials served as the source for the inoculation of triplicate Bactec 12B vials that contained concentrations of each phenothiazine that ranged from 0.0 to 50 mg/L. A separate 1:100 and 1:1000 dilution of the adjusted inoculum was made and transferred to Bactec 12B vials that contained no drug; this served as the proportional control as defined by the recommended Bactec proportional method for *M. avium* (14). The vials were incubated at 37˚C and the contents

periodically assessed by the Bactec 460 TB instrument (Becton-Dickinson Diagnostic Instrument Systems) for $^{14}CO_2$ generated from the metabolism of ^{14}C palmitic acid. The method for assessment of growth has been previously described (2, 4, 6). The MIC that completely inhibits the growth of each strain is defined for each of the phenothiazines evaluated (4).

Results

The *in vitro* activity of the phenothiazines CPZ, TZ, PMZ, PMTZ and DSP are presented in Figure 1. Briefly, the ascending order of activity, as defined by the MIC (lowest concentration that produces 100% inhibition of growth assessed by the inhibition of the $^{14}CO_2$ generated) in mg/L, was as follows: TZ (10), CPZ (25), PMZ (25-50), PMTZ (25-50) and DSP (>50). Because it has generally been assumed that the resistance of *M. avium* to drugs employed for the management of pulmonary tuberculosis produced by *M. tuberculosis* is due to the cell wall of the former, it was surprising to discover that *M. avium* is more sensitive to TZ than is *M. tuberculosis*, the latter being completed inhibited in its growth by concentrations of TZ that exceed 25 mg/L (4). However, with respect to CPZ, PMZ, PMTZ and DSP, the sensitivity of *M. avium* to these agents is practically identical to that produced against *M. tuberculosis* (4). Because the assays were conducted in triplicate and the experiments repeated at least twice, the difference between the MICs of TZ for *M. avium versus M. tuberculosis* were considered to be significant.

Discussion

Patients presenting with AIDS are at risk of acquiring a variety of opportunistic infections (15). Although each of these infections may cause death to any given AIDS patient, pulmonary infections caused by *M. avium* produce the highest mortality (15, 16) since effective drugs are few and relatively ineffective against this organism (16). Currently, azithromycin and clarithromycin are the two most effective agents for the therapy of *M. avium* pulmonary non-tubercular infections (16). However, there is now evidence that these agents produce ototoxicity (17) and arrhythmia (18), respectively. The need for effective drugs for the management of this infection is obvious.

The results obtained in our current study indicate that each of the phenothiazines studied had *in vitro* activity against the ATCC and clinical strains. The response of the reference and clinical strains was quantitatively very similar, thereby suggesting that other clinical strains will show similar responses. The order of increasing effectiveness against these strains was TZ, CPZ, PMZ, PMTZ and DSP. TZ was by far the most effective of the phenothiazines evaluated, presenting with an MIC of *ca.* 10 mg/L. Surprisingly, *M. avium* is far more sensitive to the phenothiazine TZ than are strains of *M. tuberculosis* (7, 8). Nevertheless, the MIC of 6 mg/L for TZ is not clinically achievable. However, TZ does enhance the killing of intracellular *M. tuberculosis* (10) when the concentration of this agent in the medium is of the order of 0.1 mg/L and, hence, within clinical range and below any associated with toxicity (10, 19, 20). Killing is considered to be the result of the agent being concentrated by the macrophage to levels equivalent to those that produced *in vitro* killing (10, 19, 20) and, because phenothiazines are concentrated by lysosomes (21, 22), the killing of the bacterium by macrophages that have little killing activity of their own (10) is probably due to the fusion of the phagosome with the lysosome containing the highly concentrated phenothiazine (10, 19, 20).

The greater sensitivity of *M. avium* to TZ as opposed to that produced against *M. tuberculosis* is interesting. Although at this time we cannot provide an adequate explanation for this difference, we can confidently conclude that the cell wall of *M. avium,* generally considered to be the cause for its resistance to anti-TB agents, does not lead to greater resistance to TZ.

The results of the current study are not sufficient to recommend that TZ be used for the management of AIDS patients co-infected with *M. avium*. Furthermore, even when the study that evaluated the killing activity of TZ against phagocytosed *M. avium* showed that the concentration required for such killing was comparable to that expected to be present in the plasma of patients chronically treated with this agent, the use of the mild TZ is not without risk, as shown by recent studies demonstrating that, for a very small number of patients, TZ is associated with serious arrhythmia such as "torsade de pointes" (23). However, the relationship is now in question since the number of risk factors presented by the patient may be more significant for the arrhythmia than that produced by the agent itself (24).

Conclusion

The phenothiazines CPZ, TZ, PMZ, PMTZ and DSP have *in vitro* activity against *M. avium*. TZ was the most effective of the phenothiazines, having an MIC of *ca.* 10 mg/L. Although TZ seems to be the drug of choice among the phenothiazines tested, it is not at this time recommended for the management of AIDS patients co-infected with *M. avium*.

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