Activity of the phenothiazine methdilazine alone or in combination with isoniazid or streptomycin against *Mycobacterium tuberculosis* in mice

In previous studies by our group and others, methdilazine (Md), an extensively used antihistaminic phenothiazine, demonstrated broad-spectrum antibacterial activity (Chattopadhyay *et al.*, 1988, 1998; Basu *et al.*, 2005). Md also inhibited various *Mycobacterium* spp. at 5–15 mg l⁻¹ *in vitro* and *in vivo* (Chakrabarty *et al.*, 1993). It has been reported to act by enhancement of streptomycin (Sm) or kanamycin activity against *Escherichia coli* and *Staphylococcus aureus* (Chattopadhyay *et al.*, 1988), and by damaging the bacterial cytoplasmic membrane (Chattopadhyay *et al.*, 1998) or, like other phenothiazines, by reversing clinical resistance (Kristiansen *et al.*, 2007) and inhibiting efflux pumps (Amaral *et al.*, 2008).

Patients undergoing therapy for tuberculosis are often administered Md as an antihistamine. Keeping that in mind, this study was undertaken to examine the possible effects of Md combined with the first-line antitubercular drugs isoniazid (INH) or Sm in the treatment of murine tuberculosis.

Four-week-old Swiss albino male mice were infected intravenously with 2.3×10^7 c.f.u. naturally virulent *Mycobacterium tuberculosis* (Mtb) H37Rv 102 per mouse as previously described (Dutta *et al.*, 2005, 2007), and randomly assigned to seven groups (seven mice per group): group 1, pretreatment (day 1) control; group 2, untreated control; group 3, Md treated; group 4, INH treated; group 5, Md plus INH treated; group 6, Sm treated; and group 7, Md plus Sm treated. Drugs were administered for 28 days post-infection at a dose of 10 mg (kg body weight)⁻¹ per day for Md (orally) (for the mouse the highest dose was, on a mg m⁻² basis, similar to the maximum recommended human dose of 10 mg Md per day), 25 mg (kg body weight)⁻¹ per day for INH (orally), 150 mg (kg body weight)⁻¹ per day for Sm (subcutaneously), and for Md

plus INH/Sm (INH/Sm being injected 1 h after administration of Md) at the same doses to all animals in groups 3 to 7.

Group 2 was administered 0.1 ml sterilized PBS instead of drug for those days. On the day after inoculation, seven mice (day 1 control) were sacrificed to provide the baseline values of spleen weight, and the number of c.f.u. in the lungs and spleen. The last dose of treatment was given on day 28 and all surviving mice were sacrificed on day 30 to reduce the carry-over effects of drugs in the organs. The severity of infection and the effectiveness of treatment were assessed by the survival rate, spleen weights, gross lung lesions (0, no lesions; +, fewer than 10 tubercles; ++, 10–50 tubercles; +++, more than 50 tubercles) and the c.f.u. count in the organs. The enumeration of c.f.u. from aseptically removed and homogenized lungs and spleens of sacrificed and dead mice was carried out as previously described (Dutta *et al.*, 2005, 2007).

Untreated control mice began to die from day 14, and all mice died of tuberculosis before day 28, as the mice harboured lung lesions (++ to +++), showed reduced body weight and splenomegaly (Table 1). All the mice (100%) that received both Md and INH survived for 28 days with no sign of disease. Those treated with each of Md, Sm and Md + Sm showed 71.42% survival, while those treated with INH alone showed 85.71% survival for 28 days. The gross lung pathology of treated groups reflected similar patterns (data not shown).

As indicated in Table 1, that the Md plus INH combination was more effective than usage of INH alone, and the treatment effectively prevented further development of splenomegaly caused by tuberculosis. Co-administration of Md with Sm had no effect on the c.f.u. counts of bacteria in either organ (Table 1), whereas a combination of Md and INH reduced the number of viable bacteria in lungs (1.88

log₁₀, $P < 0.05$) and spleen (0.76 log₁₀, $P < 0.05$) of the treated animals (Table 1), compared to mice treated with INH alone.

Phenothiazines are widely used in medicine for the treatment of psychosis, depression, nausea, vomiting and pruritus. The antimycobacterial properties of some phenothiazines have also been established. The antimycobacterial non-antibiotics discovered so far mostly have *in vitro* MIC values ranging from 10 to 25 mg l⁻¹ (Chakrabarty *et al.*, 1993; Dutta *et al.*, 2005, 2007; Martins *et al.*, 2007; Mazumdar *et al.*, 2009). In a published study, Md injected intraperitoneally at 10 mg kg⁻¹ daily for 1 month to mice could significantly ($P < 0.01$) protect them from challenge with (4.5×10^9 c.f.u.) Mtb H37RV (Chakrabarty *et al.*, 1993). It corroborates the reported curative activity of phenothiazine thioridazine (0.5 mg daily for 1 month) on mice infected with Mtb (10⁶ c.f.u.) (Martins *et al.*, 2007). Today it is widely discussed that phenothiazines might be considered for use as adjuvants for the management of tuberculosis infection due to their ability to kill intracellular antibiotic-sensitive/-resistant Mtb when used at concentrations in the medium well below those present in the plasma of patients treated with these agents. These concentrations *in vitro* were not toxic to the macrophage, nor did they affect *in vitro* cellular immune processes (Kristiansen *et al.*, 2007; Amaral *et al.*, 2008). The intrinsic resistance of *Mycobacterium avium* and *Mycobacterium smegmatis* is affected by antimycobacterial phenothiazine efflux pump inhibitors, such as thioridazine or chlorpromazine, an effect that might be important in the research and development of new, more effective antimycobacterial therapies (Rodrigues *et al.*, 2008). In this study, Md shows additive activity when used in combination with INH but not with Sm. Whether or not Md

Table 1. Effects of Md and/or INH/Sm on spleen weight and Mtb c.f.u. counts in organs of acutely infected mice

The results are shown as the mean $\pm$ SD (seven mice per group).

Group	Mean spleen weight (mg) $\pm$ SD	Mean c.f.u. ($\log_{10}$) $\pm$ SD	
		Lungs	Spleen
Pretreatment (1)	111.57 $\pm$ 5.53	5.06 $\pm$ 0.45	5.49 $\pm$ 0.29
Untreated (2)	648.14 $\pm$ 62.58	7.19 $\pm$ 0.40	6.11 $\pm$ 0.18
Md (3)	528.57 $\pm$ 99.08	6.32 $\pm$ 0.25	5.12 $\pm$ 0.25
INH (4)	468.85 $\pm$ 95.34	4.34 $\pm$ 0.27	3.29 $\pm$ 0.25
Sm (5)	500.57 $\pm$ 101.88	5.57 $\pm$ 0.23	4.50 $\pm$ 0.15
Md + INH (6)	384.85 $\pm$ 44.43	2.46 $\pm$ 0.55*	2.43 $\pm$ 0.21*
Md + Sm (7)	496 $\pm$ 106.84	5.45 $\pm$ 0.14	4.47 $\pm$ 0.22

*Indicates $P < 0.05$ when compared to the INH-treated group.

is detrimental when used with Sm is debatable as it is clear that there is no additive effect. This synergistic pair may impact upon the course of infection *in vivo* acting via various pathways involving immunomodulatory effects on the host. However, further investigation of the combinations in animal or actual clinical models is warranted.

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