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Title

Activity of the efflux pump inhibitor SILA 421 against drug-resistant tuberculosis

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Dear editor,

Organosilicon compounds are efflux pump inhibitors with potency as an antituberculosis drug. Of the organisilicon compounds tested, SILA 421 has been shown to have a highest potency as an antituberculosis drug (1). It shares the common pathways for antimycobacterial killing with other efflux pump inhibitors: it revealed direct in vitro activity against *M. tuberculosis* (1), it has been shown to modify resistance by inhibiting mdr-1 efflux pumps and has shown to enhance killing of *M. tuberculosis* by macrophages (1). However, the activity of SILA 421 has only been tested against the *M. tuberculosis* H37Rv type strain and one clinical XDR *M. tuberculosis* isolate so far (1). We therefore assessed the antimycobacterial activity of SILA 421 against an extended set of *M. tuberculosis* strains with varying drug susceptibility profiles. Secondly, we compared antimycobacterial activity of SILA 421 with that of thioridazine and other phenothiazines.

We selected 21 clinical *M. tuberculosis* isolates with varying drug susceptibility profiles: five isolates were pansusceptible, 11 were monoresistant (6 isoniazid monoresistant, 4 rifampin monoresistant and 1 streptomycin monoresistant) and 5 were MDR *M. tuberculosis* isolates. All isolates were extracted from a -70 °C freezer collection and sub-cultured on Ogawa medium. Inoculum preparation was performed as described previously aiming at 2 x 10^3 to 10 x 10^3 CFU (2). To determine the Minimal Inhibitory Concentration (MIC) we performed drug-susceptibility testing using a well-standardized Middlebrook 7H10 agar dilution method as described previously (2) using the following drug concentrations: 1, 2, 4, 8, and 16 mg/L. All drugs were received as chemically pure powder. SILA 421 was provided by Dr. G. Hajos (Institute for Biomolecular Chemistry, Budapest, Hungary). Chlorpromazine, desipramine, promazine and thioridazine were obtained from Sigma-Aldrich (Zwijndrecht, the Netherlands). Because the thioridazine *S*-enantiomer might induce less side-effects (4) we

studied both thioridazine enantiomers separately. The two thioridazine enantiomers were prepared by Dr. J.B. Christensen (Dept. Chemistry, Copenhagen, Denmark). All drugs were dissolved in distilled water to a stock solution of 10 g/L

The MIC of SILA 421 could be determined after a median of 14 days (range 14-18 days). The MIC_{50} was 4 mg/L and the MIC_{90} was 8 mg/L. Table 1 shows the comparison of the MIC_{50} and MIC_{90} of SILA 421 with the various phenothiazines. The MICs found for the phenothiazines were comparable with those found in the literature (3). The MIC_{50} of SILA 421 was similar to the MIC_{50} of thioridazine and chlorpromazine. On the contrary, MIC_{50} of both desipramine and promazine were both much higher than the MIC_{50} of SILA 421. When comparing the MIC_{90}, lowest MIC_{90} was measured for SILA 421.

These data show that the SILA 421 is active in vitro against *M. tuberculosis* isolates with a wide variety of drug susceptibility patterns, thereby providing incremental evidence of its antimycobacterial activity. Moreover, we showed that SILA 421 is equally active in vitro as thioridazine, another well-known efflux pump inhibitor.

In a previous study, SILA 421 has been proposed to be more potent that thioridazine (1). However, only two M. tuberculosis isolates had been tested (1). The present study confirms that SILA 421 may be a promising antituberculosis drug with activity against a wide variety of *M. tuberculosis* strains comparable to that of thioridazine. Further studies are warranted to investigate the potency of SILA 421 as an antituberculosis drug in animals and in humans.

Besides its antimycobacterial activity, SILA 421 might also have another appealing feature. Like all efflux pump inhibitors, SILA 421 will inhibit the activity of efflux pumps of MDR mycobacteria, presumptively rendering mycobacteria more susceptible to the antituberculosis drugs to which it was initially resistant as a consequence of their extrusion from the cell (5). Thus, these drug resistance modifiers might be of interest not only for their immediate

antimycobacterial activity but also as an adjunctive agent to be added to new or existing antituberculosis regimens.

Of additional interest is our finding that the *S*-enantiomer of thioridazine is as effective as thioridazine itself against *M. tuberculosis*. It is thought this *S*-enantiomer might induce less neurological side effects owing to a lower affinity to the D2 dopamine receptor (4), putatively favoring the *S*-enantiomer over the racemate as an antituberculosis drug. However, it is unknown if the D2 dopamine receptor is the only target for neurological side-effects of thioridazine and it is unclear whether the pharmacokinetic and pharmacodynamic properties of the thioridazine enantiomers are equal. Notwithstanding these limitations, our findings raise hope that structural optimization of thioridazine-derivatives is possible without losing antimycobacterial activity (3.4).

In conclusion, the present study provides confirmatory and incremental evidence of the antimycobacterial activity of SILA 421 and the phenothiazines, such as thioridazine. Therapeutic opportunities lay in the structural optimization of these drugs as well as in their drug resistance modifying effect. Future studies should bring SILA 421 and the phenothiazines further up the drug development pipeline.

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Tables

Table 1

The MIC_{50} and MIC_{90} of SILA 421 and the various phenothiazines[(a)].

Efflux pump inhibitor	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)
SILA 421	4	8	2 - 16
Thioridazine	4	16	2 - 16
Thioridazine, *S*-enantiomer	8	16	4 - 16
Thioridazine, *R*-enantiomer	8	16	4 - 16
Chlorpromazine	4	16	<1 - 16
Desipramine[(b)]	>16	>16	16 - >16
Promazine[(b)]	>16	>16	16 - >16

[(a)] MIC_{50}, MIC at which ≥ 50% of isolates are inhibited. MIC_{90}, MIC at which ≥ 90% of isolates are inhibited.

[(b)] MIC distribution was based on 20 instead of 21 isolates.