Antitubercular Activity of Disulfiram, an Antialcoholism Drug, against Multidrug- and Extensively Drug-Resistant Mycobacterium tuberculosis Isolates

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The antimycobacterial activities of disulfiram (DSF) and diethylthiocarbamate (DDC) against multidrug- and extensively drug-resistant tuberculosis (MDR/XDR-TB) clinical isolates were evaluated in vitro. Both DSF and DDC exhibited potent antitubercular activities against 42 clinical isolates of M. tuberculosis, including MDR/XDR-TB strains. Moreover, DSF showed remarkable bactericidal activity ex vivo and in vivo. Therefore, DSF might be a drug repurposed for the treatment of MDR/XDR-TB.

According to the updated guidelines of the World Health Organization, the medications effective against multidrug- and extensively drug-resistant tuberculosis (MDR/XDR-TB) are confined because only a limited selection of drugs is available; therefore, the development of novel or repurposed drugs with activity against MDR/XDR-TB is strongly desired (9). Disulfiram (DSF; tetraethyl thiuram disulfide) has been used orally in the clinical treatment of alcoholism since 1949 and has been proven to exert an inhibitory effect on aldehyde dehydrogenase in vivo with 80% bioavailability and established safety profiles (13, 33). Both DSF and its first metabolite, diethylthiocarbamate (DDC), were reported to exhibit growth-inhibitory activity against bacteria, fungi, protozoa, and viruses (2, 18, 23, 26, 27). In the mid-1950s, the tuberculostatic effects of DSF and DDC were demonstrated in vivo using guinea pigs (17). Subsequently, it was reported that DDC enhances monocyte-induced antitubercular activity in both healthy volunteers and human immunodeficiency virus-infected patients ex vivo (16). Recently, the antitubercular activities of DDC and the nitric oxide synthase inhibitor pyrrolidine dithiocarbamate (PDTC) against nonreplicating Mycobacterium tuberculosis have been demonstrated (4). In addition, we reported the unique antimycobacterial activities of dithiocarbamates and also the potent antitubercular activities of compounds containing dithiocarbamate groups, such as dimethyldithiocarbamate (DMDC), DDC, and PDTC (14, 15). More recently, the mode of action of dithiocarbamates against M. tuberculosis has been reported to be through β-class carbonic anhydrases (β-CAs), which are considered possible drug targets (19). However, the mechanism of action of DSF remains unknown.

In the present study, we evaluated the antimycobacterial activities of DSF and its metabolites against M. tuberculosis, including MDR/XDR-TB clinical isolates, in more detail. Furthermore, the intracellular bactericidal activities of these compounds against a virulent strain, M. tuberculosis H37Rv, within macrophages were examined ex vivo, and the bactericidal activity of DSF in vivo was determined using the mouse model of chronic TB. Finally, the mechanisms of action of these compounds were investigated by means of gene-overexpressing strains in vitro.

MATERIALS AND METHODS

Bacterial strains. M. tuberculosis H37Rv ATCC 25618, M. tuberculosis H37Ra ATCC 25177, Mycobacterium avium ATCC 25291, and M. avium ATCC 35718 were purchased from the American Type Culture Collection (ATCC). Mycobacterium smegmatis JATA 64-01 was provided by M. Takahashi (Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Japan). Mycobacterium bovis BCG strain Tokyo 172 (BCG Tokyo) was purchased from BCG Japan, Co. Ltd. M. avium 104 was provided by Deborah Geiman (John Hopkins University). Clinical isolates of M. tuberculosis were isolated at the Higashi Nagoya National Hospital (Japan). Mycobacterial strains were cultured in Middlebrook 7H9 broth (Difco) supplemented with 10% ADC (5% bovine serum albumin [fraction V], 2% dextrose, and 0.005% bovine liver catalase) including 0.05% Tween 80 or on Middlebrook 7H11 agar (Difco) supplemented with 10% OADC (5% bovine serum albumin [fraction V], 2% dextrose, 0.005% bovine liver catalase, and 0.05% alkaline oleic acid) at pH 6.6. Staphylococcus aureus 209PJC-1, S. aureus RN4220, S. aureus MF490, Enterococcus faecalis ATCC 19433, Enterococcus faecium ATCC 19434, Escherichia coli JM109, Klebsiella pneumoniae ATCC BAA-1705, and Pseudomonas aeruginosa PAO1 were grown on Mueller-Hinton agar (Becton, Dickinson).

Drug and reagent preparation for in vitro and in vivo studies. Isoxazid (INH), rifampin (RIF), streptomycin (STR), ethambutol (EMB),
ethionamide (ETH), p-aminosalicylic acid (PAS), ciprofloxacin (CIP), and bathocuproinedisulfonic acid disodium salt (BCPS) were purchased from Sigma-Aldrich Co. Thiamur, DSF, DMD, DDC, PDTC, and amikacin disulfate salt (AMK) were purchased from Wako Pure Chemical Industries, Ltd., Japan. Kanamycin (KAN) was purchased from Meiji Seika Kaisha, Ltd., Japan. The bulk powder of disulfiram was provided by Mitsubishi Tanabe Pharma Corporation, Japan. S-Methyl N,N-diethyldithiocarbamate (S-Me-DDC), S-methyl N,N-diethyldithiocarbamate (S-Me-DC), S-Methyl N,N-diethyldithiocarbamate (S-Me-DC), S-Me-DC, S-Me-DC sulfide, and S-Me-DC sulfone were purchased from Toronto Research Chemicals Inc., Canada. Hydrophilic or hydrophobic agents were dissolved in distilled deionized water (DDW) and dimethyl sulfoxide, respectively. Before examination, the stock solutions were diluted with assay broth, i.e., 7H9 broth. For in vivo use, RIF and DSF were dissolved or suspended in 5% gum arabic solution.

**BDT and agar dilution method.** The broth dilution test (BDT) for the determination of MICs was performed as previously described (31). The starting drug concentration was 100 μg/ml. In the cases of INH and RFP, the concentrations were 10 and 1 μg/ml, respectively. The MICs of test compounds against clinical isolates were defined as the antimicrobial concentrations that showed 90% growth inhibition of the strains. The agar dilution method for the determination of MICs using 7H11 agar at pH 6.6 was performed according to the *Manual of Clinical Microbiology* (22). The starting concentration of the drugs INH, RIF, and CIP was 3.2 μg/ml, and that of DSF, DDC, STR, EMB, KAN, and PAS was 0.125 μg/ml. The drug susceptibility test for clinical isolates was performed with the broth MIC MTB assay (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan), and the assay was conducted according to the method of the provider (32).

To determine the synergistic effects of DSF and DDC with metal ions, i.e., Zn²⁺, Cu²⁺, Co²⁺, or Al³⁺, we prepared Sauton broths with or without a metal salt, such as ZnSO₄, CuSO₄, CoCl₂, or Al₂(SO₄)₃. The 10-fold-concentrated bacterial culture was next compared to the one used for normal BDT. The MICs against several bacteria, except mycobacteria, were determined using the agar dilution method, as recommended by the Clinical and Laboratory Standards Institute (6).

**Serum bactericidal test.** The serum bactericidal test was performed according to the method of Byrne et al. (4) and the NCCLS (24). DSF, DDC, INH, and RIF were administered orally at 80 mg/kg of body weight, 80 mg/kg, 25 mg/kg, and 10 mg/kg, respectively. INH and RIF were used as positive controls. The serum bactericidal activity of the drugs was compared to that of a vehicle control, 5% gum arabic. The serum samples were collected by cardiopuncture. For DSF and DDC, serum was collected at 0.083 and 2 h after administration, in accordance with the time to the maximum concentration of DDC (0.083 h), as previously determined in our laboratory. For INH and RFP, serum was collected at 1 h after administration as previously described (4). For vehicle, serum was collected before and 2 h after administration. DDC and INH were also administered intravenously, and serum was collected at 0.883 h after administration. The pooled serum was heated at 56°C for 40 min in order to inactivate complement. Then, serum samples were filtrated using a 0.45-μm-pore-size filter followed by a 0.2-μm-pore-size syringe filter. *M. tuberculosis* H₃Rv was cultivated in 7H9 broth supplemented with 10% ADC including 0.05% Tween 80 and was grown to log phase. The final inoculum was confirmed to be 4 × 10⁶ CFU/ml by means of a colony assay using a 7H11 agar plate supplemented with 10% OADC.

**Intracellular antitubercular activities of DSF and DDC in differentiated THP-1 cells.** Cells of the human acute monocye leukemia cell line THP-1 (ATCC TIB-202) were purchased from ATCC. THP-1 cells were maintained in RPMI 1640 medium supplemented with 5% heat-inactivated fetal bovine serum, including 100 units/ml penicillin G (Meiji Seika Kaisha, Ltd., Japan) and 100 μg/ml streptomycin (Meiji Seika Kaisha, Ltd., Japan) in a humidified 5% CO₂ atmosphere at 37°C. The intracellular antitubercular assay was performed according to the method previously described (20).

**Therapeutic efficacy in an experimental mouse model of chronic TB.** In order to examine the therapeutic efficacy of DSF and to determine the therapeutic dose range, an experimental mouse model of chronic TB was used as previously described (20). Mice obtained from the Institute of Cancer Research (ICR) (*n* = 5 per group) were inoculated intravenously with 1 × 10⁶ CFU/ml of *M. tuberculosis* H₃Rv through the caudal vein, and the infection was allowed to develop for 28 days. The test compounds were then administered orally once daily for 28 days (RIF, 5 to 20 mg/kg; DSF, 40 to 160 mg/kg [2-fold dilutions]). The extracted lungs and spleen were homogenized with 1 ml DDW. The bacterial burden (CFU/organisms) in either organ was counted using 7H11 agar plates to determine the therapeutic efficacy. Statistical analysis was conducted using Microsoft Office Excel 2007 software. Statistical significance was set at *P* values of <0.05, <0.01, and <0.001 compared to the vehicle control group.

**Sulfonation mechanisms of DSF and DDC.** *ethA* or *ethR*-overexpressing strains were prepared as described previously and were used for MIC determination (1, 7).

**RESULTS AND DISCUSSION**

In accordance with previous reports, DSF exhibited antitubercular activity, with MICs of 1.56 to 3.13 μg/ml. Despite the use of conditions similar to those in a previous study (4), DSF exhibited potent antitubercular activity, with an MIC of 1.56 μg/ml at pH 6.6 using Middlebrook 7H9 broth (Table 1). Importantly, the MIC of the bulk powder of DSF provided by Mitsubishi Tanabe Pharma, which is generally used in the clinical treatment of alcoholism in Japan, was comparable to that of chemical reagent DSF (data not shown). Additionally, the MIC of DSF against BCG Tokyo using 7H11 agar plates was also equivalent to the MICs of anti-TB drugs, i.e., EMB and KAN (MICs = 1 to 4 μg/ml) (see Table S1 in the supplemental material). Among mycobacterial species, although the antibacterial spectrum of DSF is broad, the antitubercular activity of DDC was highly specific to slow-
including MDR/XDR-TB strains (Table 2). The MIC90s of DSF and DDC against more than 40 clinical isolates of M. tuberculosis were determined by BDT (see Table S2 in the supplemental material) (28). The results revealed that MICs of DSF against other Gram-positive bacteria, such as S. aureus (MIC = 16 μg/ml), E. faecalis (MIC = 32 μg/ml), and E. faecium (MIC = 32 μg/ml), was similar to that of DDC (see Table S2 in the supplemental material). As previously reported, unlike DSF, DDC exhibited antibacterial activity, with MICs of 128 μg/ml against Gram-negative bacteria, i.e., E. coli, K. pneumoniae, and P. aeruginosa (see Table S2 in the supplemental material) (28).

DSF is well-known to be a prodrug and is enzymatically metabolized to DDC in blood, followed by activation owing to certain reactions, such as S-methylation, oxidation, and sulfonation (1). The antimycobacterial activities of the metabolites of DSF, i.e., S-Me-DSF, S-Me-DDC, S-Me-DTC sulfoxide, and S-Me-DTC sulfone, were determined by BDT in vitro. The results revealed that S-methylation of the sulfhydryl group of DSF led to deactivation (MIC > 100 μg/ml), in spite of its oxidation, and the metabolites obtained by sulfonation had partially restored antimycobacterial activities (MICs = 12.5 to 25 μg/ml) (Table 1). Therefore, the results suggest that not only DSF but also its metabolites, namely, DDC, S-Me-DTC sulfoxide, and S-Me-DTC sulfone, are biologically active in the human body, which complicated the assessment of the antitubercular activity of DSF after oral administration.

We next further determined the antitubercular activities of DSF and DDC against clinical isolates of M. tuberculosis in vitro. As expected, these compounds exhibited potent antitubercular activities against more than 40 clinical isolates of M. tuberculosis, including MDR/XDR-TB strains (Table 2). The MIC90s of DSF and DDC against clinical isolates were 1.56 μg/ml and 3.13 μg/ml, respectively (Table 2). Importantly, there was no cross-resistance of DSF or DDC to the currently available anti-TB drugs, including fluoroquinolones such as levofloxacin, sparfloxacin, and CIP (see Table S3 in the supplemental material). Thereby, DSF and DDC may be implemented in future pharmacological regimens against MDR/XDR-TB.

It has been reported that DDC enhances monocyte-induced antitubercular activity ex vivo (16). Therefore, in order to confirm whether DSF and its metabolites are effective within macrophages, we determined the bactericidal activities of these compounds against intracellular M. tuberculosis in differentiated THP-1 cells. As shown in Fig. 1, these compounds exhibited potent bactericidal activities at 6 to 30 μg/ml and 10 to 30 μg/ml, respectively, in a dose-dependent manner, unlike STR and the bacteriostatic drug EMB. Likewise, S-Me-DTC sulfone, the active metabolite of DSF, exhibited intracellular bactericidal activity at 30 μg/ml, but S-Me-DTC did not, in agreement with the results presented in Table 1 (Fig. 1). These data indicate that DSF, DDC, and S-oxidized metabolites, especially S-Me-DTC sulfone, are effective against intracellular M. tuberculosis.

Subsequently, we examined whether DSF and DDC exhibited bactericidal activity in vivo by means of a serum bactericidal test. The serum bactericidal activities of INH and RIF were significant at a titer of 1:32 compared to the activity of the vehicle control. As expected, the serum sample collected at 2 h after DSF administration orally exhibited bactericidal activity at a titer of 1:2, which was comparable to that of DDC administered intravenously, whereas the serum sample collected at 0.083 h after administration exhibited less activity (Table 3). Likewise, the serum sample collected at 2 h after DDC administration orally exhibited bactericidal activity, and the serum sample collected at 0.083 h exhibited less activity at a titer of 1:8; the serum sample from mice treated orally with DSF, but not DDC, exhibited bactericidal activity. Therefore, DSF has more potent bactericidal activity than DDC (Table 3). These results also indicated that DDC exhibits bactericidal activity in vivo.

Previously, the tuberculostatic effects of dithiocarbamates and thiuram disulfides have been examined in experimental tuberculosis of guinea pigs (n = 10) (17). Whereas this report is considerably important for the development of drugs with activity against TB, only evidence based on pathological findings without bacteriological examination of the lungs had been shown. This prompted us to evaluate the bactericidal activity of DSF in vivo in the mouse model of chronic TB. In the group administered DSF, significant reductions in the numbers of CFU were observed in both lungs and spleen at 0 to 160 mg/kg (P < 0.01) compared to the numbers of CFU for the vehicle control group (Fig. 2). The reduction in the numbers of pulmonary CFU by DSF at 80 mg/kg was similar to that by RIF at 10 mg/kg (Fig. 2). Whereas it has been shown that the antitubercular activity of RIF is not as strong as that of INH, RIF concentrations of 10 mg/kg were sufficient to reduce the number of pulmonary CFU. This result demonstrated that DSF has significant antitubercular activity as a single drug and can be used as a single drug in the treatment of TB. Furthermore, the reduction in the numbers of pulmonary CFU by DSF was more effective than that by DDC at 80 mg/kg (Fig. 2).

### Table 2 Antitubercular activities of DSF and DDC against drug-susceptible and -resistant clinical isolates of M. tuberculosis

<table>
<thead>
<tr>
<th>Clinical isolate</th>
<th>No. of strains</th>
<th>Agent</th>
<th>MIC (μg/ml)</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS-TB</td>
<td>20</td>
<td>DSF</td>
<td>0.78–1.56</td>
<td>0.78</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DDC</td>
<td>1.56–3.13</td>
<td>1.56</td>
<td>3.13</td>
</tr>
<tr>
<td>DR-TB</td>
<td>22</td>
<td>DSF</td>
<td>0.78–1.56</td>
<td>1.56</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DDC</td>
<td>1.56–6.25</td>
<td>3.13</td>
<td>3.13</td>
</tr>
</tbody>
</table>

a Experiments were performed in duplicate. These results are representative of two separate experiments. DS-TB, drug-susceptible TB; DR-TB, drug-resistant TB; DSF, disulfiram; DDC, diethyldithiocarbamate.

b DR-TB includes multidrug-resistant TB (n = 13) and extensively drug-resistant TB (n = 5). Drug resistance profiles are depicted in Table S3 in the supplemental material.
reported that DSF possessed a tuberculostatic effect at 20 mg/kg in the experimental model of tuberculosis in guinea pigs, there was no significant bactericidal activity in either the lungs or spleens of mice treated with DSF at 40 mg/kg (Fig. 2) (17). Taken together, these results suggest that DSF would exhibit a therapeutic effect against \textit{M. tuberculosis} infection. According to previous reports, DSF could be administered at 6 g without considerable harm to humans, and intriguingly, it has been demonstrated that DSF and its metabolites preferentially transfer to lungs rather than to plasma, brain, or liver, suggesting that the use of DSF is feasible for the treatment of TB, especially for MDR/XDR-TB (8, 29).

The currently available anti-TB drugs, i.e., isoniazid, streptomycin, ethambutol, and \textit{p}-aminosalicylic acid, have hitherto been known to be metal chelators (10, 11, 12). Intriguingly, for example, it has been reported that PAS may exert a synergistic effect with copper ion on antitubercular activity (11a). \textbf{β}-CAs, reported to be the drug target of dithiocarbamates, belong to the metalloenzyme family and include Zn ion at the active site when the enzymatic reaction is catalyzed (19). On the other hand, dithiocarbamates, e.g., DDC and PDTC, have been known to be metal chelators and have been reported to possess synergistic effects with copper ion on killing activity against protozoa, i.e., \textit{Plasmodium falciparum} and \textit{Toxoplasma gondii} (5, 21). Thus, to assess whether metal ions, namely, Zn\textsuperscript{2+}, Cu\textsuperscript{2+}, Co\textsuperscript{2+}, or Al\textsuperscript{3+}, affect the antitubercular activities of DSF and DDC, we prepared metal ion-containing Sauton broth medium and determined the MICs of these compounds. Increased antitubercular activities of these compounds were observed in CuSO\textsubscript{4}-containing broth in a dose-dependent manner, unlike the findings for broth containing ZnSO\textsubscript{4}, CoCl\textsubscript{2}, or Al\textsubscript{2}(SO\textsubscript{4})\textsubscript{3}, indicating that the antitubercular activities of these compounds depend on small amounts of copper ion (see Table S4 in the supplemental material). Consequently, these results indicate that DSF and DDC exert synergistic effects with copper ion (\textit{>1 nM}), unlike Zn\textsuperscript{2+}, Co\textsuperscript{2+}, or Al\textsuperscript{3+}. For this roundup, the antitubercular activities of these compounds were determined using the copper ion chelator BCPS. BCPS was reported to inhibit the activation of signal transduction and the cell growth activated by DSF in melanoma via chelating copper ion (3). As expected, the antitubercular activities of these compounds were suppressed by BCPS in 7H9 broth, normally supplemented with 4 \mu M Cu\textsuperscript{2+} ion, and similar results were obtained for INH and ETH (Table 4). These results suggest that these compounds possess synergistic effects with the small amount of copper ion existing in the human body (30).

Based on our knowledge, \textbf{β}-CAs have been reported to be the drug targets of sulfonamides and sulfamates (25). Thereby, we considered that dithiocarbamates required sulfonation via intracellular enzymes, e.g., monooxygenase (EhA), for activation (1, 7). Meanwhile, DDC is catalyzed by the monooxygenase CYP2E1 in the human body. Hence, we hypothesized that DDC may possess a mechanism of activation that relies on the monooxygenase EhA and studied whether \textit{ethA} expression was required for the

\begin{table}
\centering
\caption{Serum bactericidal activities of each agent compared to vehicle control\textsuperscript{a}}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
 & \multicolumn{2}{|c|}{DSF, p.o.} & \multicolumn{2}{|c|}{DDC, p.o.} & \multicolumn{2}{|c|}{INH, p.o., 1 h} & \multicolumn{2}{|c|}{RIF, p.o., 1 h} & \multicolumn{2}{|c|}{DDC, i.v., 0.083 h} & \multicolumn{2}{|c|}{INH, i.v., 0.083 h} \\
Titer & 0.083 h & 2 h & 0.083 h & 2 h & 1 h & 1 h & 0.083 h & 0.083 h & 0.083 h & 0.083 h & 0.083 h & 0.083 h \\
\hline
1:2 & 1.84 ± 0.03 & 2.66 ± 0.01 & 1.93 ± 0.05 & 2.46 ± 0.01 & 4.60 ± 0 & ND & 2.63 ± 0.03 & ND & 2.63 ± 0.03 & ND & 2.63 ± 0.03 & ND \\
1:4 & 1.55 ± 0.003 & 1.79 ± 0.11 & 1.57 ± 0.04 & 1.58 ± 0.05 & 4.22 ± 0.12 & 4.600 & 1.90 ± 0.08 & 4.30 ± 0.43 & 2.16 ± 0.06 & 3.72 ± 0.16 & 3.72 ± 0.16 & 3.72 ± 0.16 \\
1:8 & 1.41 ± 0.01 & 1.49 ± 0.1 & — & — & 3.57 ± 0.06 & 3.85 ± 0.21 & 1.36 ± 0.07 & 3.72 ± 0.16 & 3.72 ± 0.16 & 3.72 ± 0.16 & 3.72 ± 0.16 & 3.72 ± 0.16 \\
1:16 & — & — & — & — & 2.83 ± 0.09 & 3.58 ± 0.03 & 2.83 ± 0.09 & 3.72 ± 0.16 & 3.72 ± 0.16 & 3.72 ± 0.16 & 3.72 ± 0.16 & 3.72 ± 0.16 \\
1:32 & — & — & — & — & 2.32 ± 0.02 & 2.68 ± 0.03 & 2.32 ± 0.02 & 3.72 ± 0.16 & 3.72 ± 0.16 & 3.72 ± 0.16 & 3.72 ± 0.16 & 3.72 ± 0.16 \\
\hline
\end{tabular}
\textsuperscript{a}Each value (sample–vehicle control) represents the mean ± SD (n = 3). ND, not detected (>5.0 log\textsubscript{10} CFU/ml); —, <1.0 log\textsubscript{10} CFU/ml; DSF, disulfiram; DDC, diethyldithiocarbamate; INH, isoniazid; RIF, rifampin; p.o., per os; i.v., intravenous injection.
\end{table}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Therapeutic effects of rifampin (R) and disulfiram (D) in an experimental mouse model of chronic TB. ICR mice were inoculated intravenously with \textit{M. tuberculosis} \textit{H}\textsubscript{3}\text{Rv}. After 28 days, test compounds were administered orally once daily for 28 days, and then lungs and spleen were extracted and the numbers of colonies in organs (log\textsubscript{10} CFU/organ) were determined using 7H11 agar plates. Rifampin was used at concentrations from 5 to 20 mg/kg, disulfiram was used at concentrations from 40 to 160 mg/kg, and vehicle (5% arabic gum) alone was administered as a control. Error bars represent means ± SDs (n = 5). The Student \textit{t} test was used to compare different treatment groups. Statistical significance was set at \textit{P} values of <0.05 (*), <0.01 (**), and <0.001 (***).}
\end{figure}
TABLE 4 MICs of thiuram disulfides, dithiocarbamates, and antitubercular drugs against M. tuberculosis with or without copper chelator

<table>
<thead>
<tr>
<th>Agent</th>
<th>MIC (μg/ml) using 7H9 broth with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No BCPS</td>
</tr>
<tr>
<td>Thiuram disulfide</td>
<td></td>
</tr>
<tr>
<td>Thiuram</td>
<td>0.78</td>
</tr>
<tr>
<td>DSF</td>
<td>1.56</td>
</tr>
<tr>
<td>Dithiocarbamate</td>
<td></td>
</tr>
<tr>
<td>DMDC</td>
<td>1.56</td>
</tr>
<tr>
<td>DDC</td>
<td>3.13</td>
</tr>
<tr>
<td>PTDTC</td>
<td>0.4</td>
</tr>
<tr>
<td>Anti-TB drugs</td>
<td></td>
</tr>
<tr>
<td>INH</td>
<td>0.08</td>
</tr>
<tr>
<td>RIF</td>
<td>0.002</td>
</tr>
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<td>STR</td>
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<td>EMB</td>
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<tr>
<td>ETH</td>
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</tr>
<tr>
<td>CIP</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*Experiments were performed in duplicate. These results are representative of three separate experiments. DSF, disulfiram; DMDC, dimethyldithiocarbamate; DDC, diethyldithiocarbamate; PTDTC, pyrrolidine dithiocarbamate; INH, isoniazid; RIF, rifampin; STR, streptomycin; EMB, ethambutol; ETH, ethionamide; CIP, ciprofloxacin; BCPS, bathocuproinedisulfonic acid.

antimycobacterial activities of DSF and DDC. As control results, the ethR-overexpressing strain exhibited high levels of resistance to ETH, whereas the ethA-overexpressing strain was hypersusceptible to ETH (see Table S5 in the supplemental material). However, the MICs of both DSF and DDC against either the ethR- or ethA-overexpressing strain were equivalent to those against the wild-type strain (BCG pMV261), indicating that the antimycobacterial activities of these compounds do not depend on ethR expression (see Table S5 in the supplemental material). Albeit the mechanisms of sulfonation of these compounds are currently unknown, this mechanism via monoxygenase must be necessary to exert the antimycobacterial activities.

In conclusion, DSF is effective against MDR/XDR-TB, exhibits bactericidal activity within macrophages, and kills M. tuberculosis in mice, indicating that DSF might be a drug that may be repurposed for the treatment of MDR/XDR-TB. Further investigation on the mechanisms of action is now required to identify the potent drug targets, which will be important for the design of novel anti-tubercular drugs.

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