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Anthelmintic Avermectins Kill *Mycobacterium tuberculosis*, Including Multidrug-Resistant Clinical Strains

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Avermectins are a family of macrolides known for their anthelmintic activities and traditionally believed to be inactive against all bacteria. Here we report that members of the family, ivermectin, selamectin, and moxidectin, are bactericidal against mycobacterial species, including multidrug-resistant and extensively drug-resistant clinical strains of *Mycobacterium tuberculosis*. Avermectins are approved for clinical and veterinary uses and have documented pharmacokinetic and safety profiles. We suggest that avermectins could be repurposed for tuberculosis treatment.

Antibacterial drug discovery is a costly endeavor with a very limited probability of success (1). The need for new therapies is especially acute in the case of tuberculosis (TB). While *Mycobacterium tuberculosis* is notoriously resistant to most antibiotics and new drugs with novel modes of action are urgently needed, such compounds have proven to be rare and difficult to identify (2). An alternative approach to generate new TB treatment options in a timely and cost-effective manner is "repurposing," i.e., identifying new applications for existing, clinically approved drugs (alone or in combination) with known pharmaceutical properties (3). In a recent study, we validated the concept that drugs that do not inhibit *M. tuberculosis* at clinically relevant concentrations might be introduced for TB therapy if they could be administered within a synergistic combination (4).

In the course of this screening program, we found that selamectin, a commonly used anthelminthic veterinary drug of the avermectin family, effectively inhibited mycobacterial growth in agar and liquid cultures. The avermectins were discovered in the mid-1970s in an antinematode screening program led by Kitasato Institute and the Merck, Sharp, and Dohme (MSD) laboratories (5). The antimycobacterial activity we discovered was surprising because the avermectins were thought to be only effective against helminths, insects, and arachnids and to be inactive against flatworms, protozoa, bacteria, and fungi (5–9). However, we could not identify literature with specific information describing the (lack of) antibacterial activity of the avermectins. In all probability, these negative data remained proprietary information and were never published. We therefore examined the antibacterial effectiveness of four avermectins (doramectin, ivermectin, moxidectin, and selamectin) (Fig. 1) against representative Gram-positive and Gram-negative bacteria (Table 1). Inhibitory effects were not observed on any of these bacteria at concentrations as high as 256 μg/ml using the bacterial growth indicator MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]. The avermectins were then tested for their inhibitory activities against various *Mycobacterium* species using the same MTT assay (Table 1). This assay has been previously used to test drug sensitivity of *M. tuberculosis*, and the results are entirely consistent with other well-established methods based on nitrate reductase (12), resazurin (12), [^{3}H]glycerol uptake (13), or the gold standard proportion method of viable CFU after treatment (12, 14). All four avermectins inhibited growth of *Mycobacterium bovis* BCG and *M. tuberculosis* laboratory strains (H37Rv, CDC 1551, and Erdman) at concentrations ranging from 1 to 8 μg/ml. Three of the four also inhibited growth of *Mycobacterium smegmatis* within this concentration range. All four avermectins were less active against *Mycobacterium avium*; doramectin had lower levels of activity against all *Mycobacterium* species. Recent reports have shown that the antimycobacterial activity of some drugs is dependent on the presence of glycerol in the assay medium, leading to the identification of leads that lack activity *in vivo* (15, 16). When the activities of ivermectin, moxidectin, and selamectin were assayed in the absence of glycerol against *M. tuberculosis*, only a slight decrease (twofold) in their MICs was observed, demonstrating a glycerol-independent mode of action for the avermectins. In summary, the avermectins were found to be active against each of the four *Mycobacterium* species tested, while all were inactive against bacteria belonging to diverse related and unrelated taxa. Although these structurally related compounds had detectable differences in their activities against the four *Mycobacterium* species, our results suggest that mycobacteria share an unknown essential target for avermectins.

In order to investigate the potential inclusion of avermectins in the limited repertoire of TB drugs that might be used against drug-resistant strains, the activities of avermectins were also surveyed against multidrug-resistant (MDR) and extensively drug-resistant (XDR) *M. tuberculosis* clinical isolates from different geographical locations (Table 1). The MICs of ivermectin, selamectin, and moxidectin were similar against a panel of 27 MDR and XDR clinical isolates having elevated drug resistance profiles including first- and second-line anti-TB drugs, such as ethambutol, ethionamide, isoniazid, kanamycin, rifabutin, rifampin, *p*-amino salicylate (PAS), pyrazinamide, and streptomycin (Table 1). Only three multidrug-resistant (CI15072, CI12081, and BC-MDR2) and two

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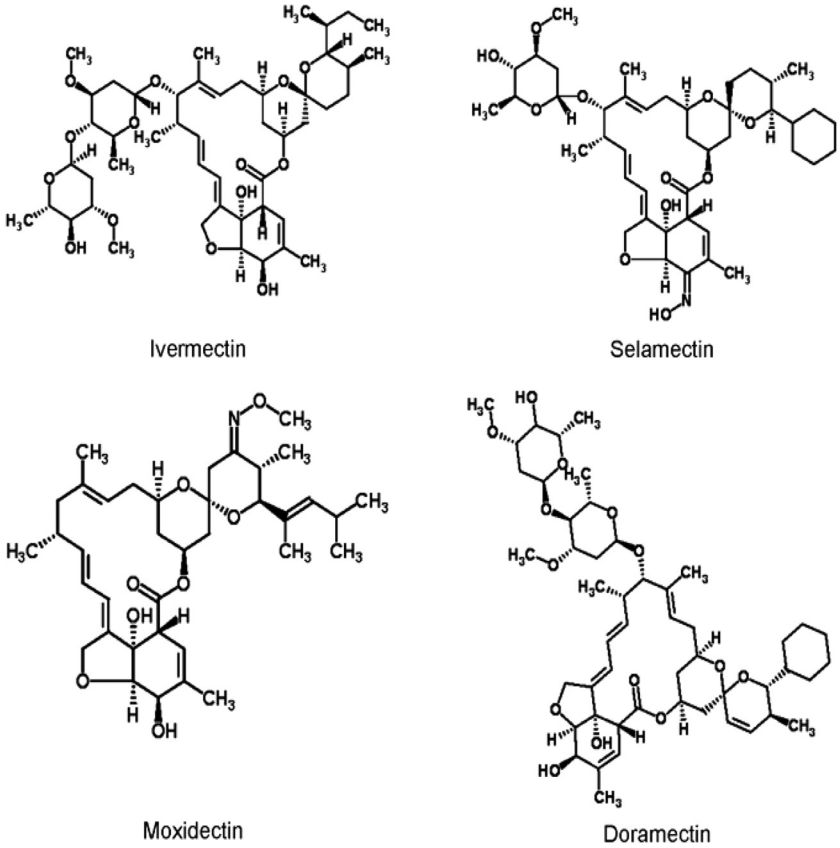

FIG 1 Avermectins used in this study. Images were obtained from ChemSpider.

drug-susceptible (BC-DS4 and BC-DS5) strains were less sensitive to ivermectin ($MIC_{90} > 24$ μg/ml). Nevertheless, inhibitory activity against these strains was reflected in low MIC_{50} values (<8 μg/ml). Importantly, the sensitivity of these strains to selamectin and moxidectin was unaffected. In summary, the avermectins were as effective against most drug-resistant *M. tuberculosis* clinical isolates as they were against *M. tuberculosis* laboratory strains.

To address the question of whether avermectins are bactericidal or bacteriostatic, survival kinetic experiments were done for ivermectin, selamectin, and moxidectin (Fig. 2). Two experiments performed independently, under similar but not identical growth conditions (see the Fig. 2 legend), measured kill kinetics. In the first experiment (Fig. 2A), 21-day kill curves were performed using various concentrations of ivermectin, selamectin, and moxidectin against the laboratory *M. tuberculosis* strain H37Rv. Here, selamectin showed the strongest bactericidal profile. All avermectins proved to be bactericidal, reducing initial bacterial viability up to 6 orders of magnitude (the limits of CFU detection). In the comparable kill kinetic experiment (Fig. 2B) performed independently at another location, the activity of the same dose (20 μg/ml) of each of the avermectins was measured over the same time period against *M. tuberculosis* H37Rv and $mc^2$5857 (an MDR strain; see Table 1 for details). Results were consistent with those described above (Fig. 2A). Moreover, all avermectins tested showed promising bactericidal activity against the MDR strain, reinforcing the MIC data and suggesting potential application of the avermectins for the treatment of MDR and XDR TB patients.

The *in vitro* pharmacodynamic parameters of the avermectins were further analyzed in a third experiment (Fig. 3). *In vitro* dose-response curves for the avermectins were obtained by plotting the change in $\log_{10}$ CFU of the inoculum against the drug concentration *C*/MIC. Using an alternative method to visualize kill kinetics, each avermectin concentration was multiplied by the time of exposure ($C \times T_{days}$) and then divided by the MIC to give the *in vitro* area under the concentration-time curve (AUC/MIC ratio), a standard measure of drug exposure. These analyses both showed that avermectins had exposure-dependent kill kinetics against *M. tuberculosis* under standard *in vitro* broth conditions. The AUC/MIC needed to achieve 1 $\log_{10}$ CFU/ml reduction varied between 2 and 4, while a bactericidal effect (4 $\log_{10}$ CFU/ml reduction, 99.99% killing) required AUC/MIC ratios between 12 and 14. From a TB drug treatment perspective, this value indicates the strong bactericidal effect of avermectins relative to rifampin, the standard frontline drug. The AUC at 24 h (AUC_{24})/MIC value required for a comparable 1 $\log_{10}$ CFU/ml reduction of the first-line antituberculosis drug rifampin is more than 10 times higher (18).

Ivermectin has been used to treat human onchocerciasis and lymphatic filariasis for over 20 years (19–21). Other avermectins (doramectin, moxidectin, and selamectin) used in veterinary medicine for nematode control in pets and livestock are known to be safe and well-tolerated for these indications (19). Selamectin can be administered topically, subcutaneously, or orally in the

TABLE 1 Antimicrobial activities of four avermectins against bacterial species, including multidrug-resistant *M. tuberculosis* clinical isolates[a]

Species	Strain[b]	Drug resistance profile[c]	*In vitro* MIC_{90} (μg/ml) for:			
			Ivermectin	Selamectin	Moxidectin	Doramectin[d]
Escherichia coli	O157:H7	WT	>256	>256	>256	>256
Acinetobacter baumannii	ATCC 1960	WT	>256	>256	>256	>256
Pseudomonas aeruginosa	PA01 (H103)	WT	>256	>256	>256	>256
S. lividans	1326	WT	>256	>256	>256	>256
R. jostii	RHA1	WT	>256	>256	>256	>256
Kocuria rhizophila		WT	>256	>256	>256	>256
Staphylococcus aureus	ATCC 25923	WT	>256	>256	>256	>256
M. smegmatis	mc^2155	WT	8	4	4	128
M. avium	ATCC 25291	WT	>128	16	>128	>128
M. bovis	BCG Pasteur	WT	4	4	4	8
M. tuberculosis	H37Rv	WT	6	3	3	8
M. tuberculosis	CDC 1551	WT	4–8	1	2	4–8
M. tuberculosis	Erdman	WT	8	2	2–4	8
M. tuberculosis	1254		8	2–4	4	8–16
M. tuberculosis	H37Rv mc^24977	INH	6	1.5	3	ND
M. tuberculosis	CI5447	INH	1.5	1.5	0.8–1.5	ND
M. tuberculosis	CI5297	INH	6	3	6	ND
M. tuberculosis	CI5305	INH	3	1.5	1.5	ND
M. tuberculosis	H37Rv mc^25857	INH, RIF	3	1.5	3	ND
M. tuberculosis	H37Rv mc^25858	INH, RIF	3	1.5	3	ND
M. tuberculosis	V2475	INH, RIF	1.5	1.5	3	ND
M. tuberculosis	CI5058	INH, RIF	6	6	6	ND
M. tuberculosis	CI5324	INH, RIF	6	3	3	ND
M. tuberculosis	CI5400	INH, RIF	3	3	3	ND
M. tuberculosis	CI5072	INH, RIF, SM	>24	3–6	3	ND
M. tuberculosis	CI5358	INH, EMB, ETH	3	1.5	3	ND
M. tuberculosis	CI5459	INH, EMB, ETH	3	1.5–3	1.5	ND
M. tuberculosis	CI12081	INH, RIF, SM, EMB, ETH	>24	3	3	ND
M. tuberculosis	KZN11	INH, RIF, SM, EMB	3	3	3	ND
M. tuberculosis	KZN5	INH, RIF, SM, EMB, ETH, KM, PZA	6	3	3	ND
M. tuberculosis	KZN6	INH, RIF, SM, EMB, ETH, KM, PZA	6	6	3	ND
M. tuberculosis	KZN12	INH, RIF, SM, EMB, ETH, KM, PZA	3	3	1.5	ND
M. tuberculosis	KZN14	INH, RIF, SM, EMB, ETH, KM, PZA	6–12	6	3–6	ND
M. tuberculosis	KZN15	INH, RIF, SM, EMB, ETH, KM, PZA	6	3	3	ND
M. tuberculosis	KZN16	INH, RIF, SM, EMB, ETH, KM, PZA	3	1.5	1.5	ND
M. tuberculosis	BC-DS1		4–16	2	4	ND
M. tuberculosis	BC-DS3		8–16	1–2	2	ND
M. tuberculosis	BC-DS4		64 (8)	1–2	2	ND
M. tuberculosis	BC-DS5		>128 (8)	2–4	2–4	ND
M. tuberculosis	BC-MDR2	INH, RIF, PZA, SM, RBT	>128 (8)	2	2–4	ND
M. tuberculosis	BC-MDR3	INH, RIF, RBT	1–2	0.5–1	1–2	ND
M. tuberculosis	BC-MDR4	INH, RIF, EMB, PZA, RBT	4–16	2	2–4	ND
M. tuberculosis	BC-MDR5	INH, RIF, PZA, SM, PAS, RBT	4–16	2	2–4	ND

[a] Mycobacterial strains were assayed in liquid 7H9 media containing 0.2% glycerol and 10% albumin-dextrose-saline. Nonmycobacterial strains were assayed in LB medium. The MTT assay was used to determine the concentration that inhibited growth by 90%. Values in parentheses indicate 50% growth inhibition (MIC_{50}). The *M. smegmatis* strain was incubated for 3 days, the *M. avium*, *M. bovis* BCG, and *M. tuberculosis* strains were incubated for 7 days, and the nonmycobacterial strains were incubated overnight before the addition of MTT.

[b] Strains V2475 and 1254 have been described previously (10, 11). CI strains are clinical isolates from Mexico. KZN strains are clinical isolates from KwaZulu-Natal, South Africa. BC strains are clinical isolates from British Columbia, Canada.

[c] WT, wild type (no resistance mutations); EMB, ethambutol; ETH, ethionamide; INH, isoniazid; KM, kanamycin; RBT, rifabutin; RIF; rifampin; PAS, *p*-amino salicylate; PZA, pyrazinamide; SM, streptomycin.

[d] ND, not determined.

veterinary setting to treat a number of ecto- and endoparasite conditions in dogs and cats, and it is not presently approved for human use (22). Moxidectin is also safe in humans (23), and it is currently undergoing a phase III clinical trial to compare its efficacy with ivermectin in subjects with *Onchocerca volvulus* infection (http://clinicaltrials.gov/ct2/show/NCT00790998). Because ivermectin is extremely well-tolerated, effective, orally active, and associated with long-term safety, Merck & Co. has donated it to patients with river blindness in needy areas throughout the world (24). Furthermore, the success of this longstanding give-away program indicates that industrial production processes are established and ivermectin could be distributed to large populations with high incidences of MDR TB, a distinct advantage for implementation of TB drug therapy programs. However, to introduce

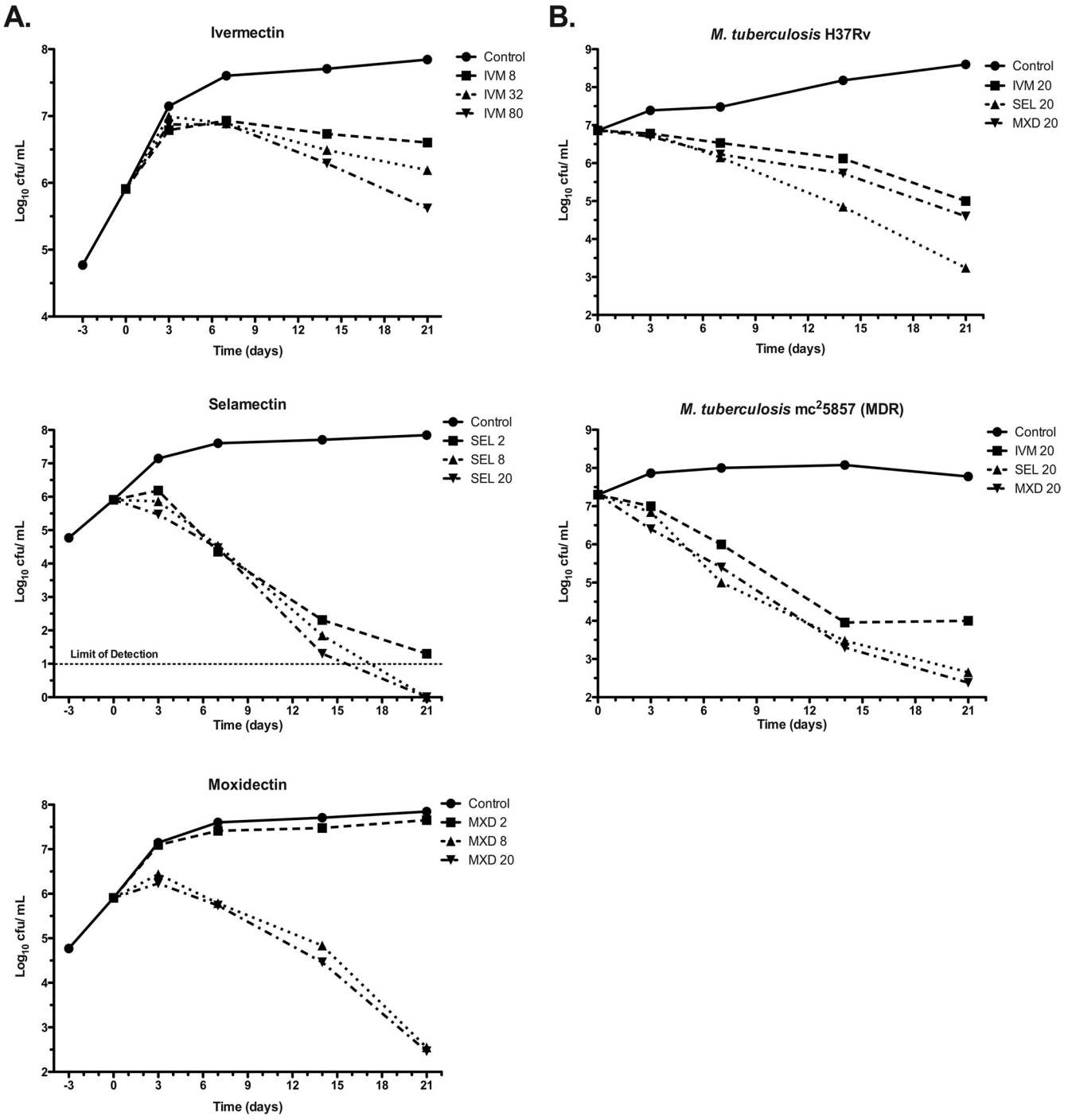

FIG 2 Time-kill kinetics of avermectins against *M. tuberculosis*. Two experiments were performed independently, at two different locations, under similar but not identical growth conditions. (A) Dose titration. Frozen stocks of *M. tuberculosis* H37Rv were cultured in 15 ml of 7H9 broth supplemented with 10% albumin-dextrose-catalase, 0.2% glycerol in standing 25-cm^2 tissue culture flasks at 37°C and 5% CO_2 for 3 days before the addition of avermectins. Cultures were agitated only during sampling. (B) Fixed dose strain comparison. Cultures (5 ml) of *M. tuberculosis* H37Rv and mc^25857 (an MDR isolate) were pregrown in shaking 35-ml bottles in 7H9 broth supplemented with 10% ADC, 0.2% glycerol, and 0.05% tyloxapol to an optical density at 600 nm (OD_{600}) of 0.8. To assess drug activity, cultures were washed once in phosphate-buffered saline (PBS), diluted (1/150) in the same medium (without tyloxapol), and grown at 37°C in shaking 15-ml conical tubes containing ivermectin, selamectin, or moxidectin at 20 μg/ml. Prior to plating, cell clumps were disrupted by sonication and viability was quantified by plating on 7H10 agar supplemented with 10% oleic acid-albumin-dextrose-catalase and 0.2% glycerol. Plates were incubated at 37°C, and colonies were counted after 2 weeks of incubation (microscopically) and reassessed after 4 weeks. Concentrations of avermectins are expressed in μg/ml. IVM, ivermectin; SEL, selamectin; and MXD, moxidectin.

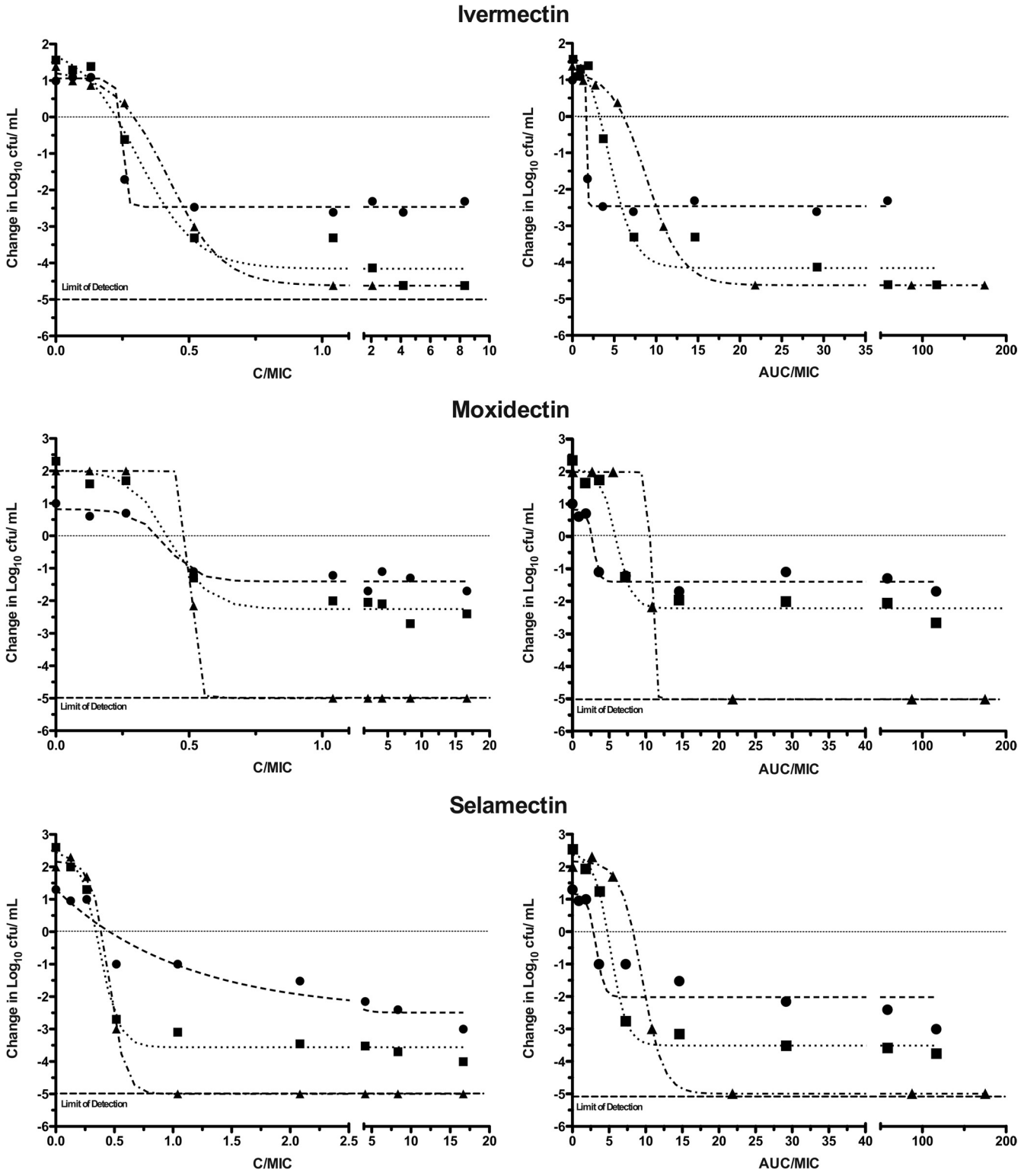

FIG 3 Effect of concentration and exposure on the bactericidal activities of avermectins against *M. tuberculosis*. Comparison of concentration and area under the curve over MIC ratios (C/MIC and AUC/MIC, respectively) on the bactericidal activities of the avermectins. The bactericidal effect was calculated on the basis of the initial inoculum prior to the addition of avermectins. Analysis of antimycobacterial activity was performed by comparing the rates of bacterial killing determined by nonlinear regression analysis with 95% confidence limits. The r^2 values on days 7 (filled circles), 14 (filled squares), and 21 (filled triangles) were 0.9959, 0.9766, and 0.9989 for ivermectin, 0.9591, 0.9827, and not available for moxidectin, and 0.88, 0.9949, and 0.9992 for selamectin, respectively. Cells were processed as described in the Fig. 2 legend for panel B but grown at 37°C in shaking 96-well plates containing 2-fold serial drug dilutions. The MIC values used for calculations were 6, 3, and 3 μg/ml for ivermectin, moxidectin, and selamectin, respectively, as calculated by the MTT assay.

any new TB therapy (especially for MDR or XDR TB), it is essential to establish that it is equal to or better than current treatment options; the promising 2- to 8-μg/ml range of MICs for avermectins in liquid cultures is comparable to that of second-line TB drugs, ranging from 1 to 25 μg/ml against *M. tuberculosis* H37Rv (25). The *in vitro* AUC/MIC ratios of avermectins are a pharmacodynamic predictor of effective bactericidal activity *in vivo*. Importantly, the low exposure needed to achieve this effect (AUC/MIC ratios of 10 to 15) indicates the potential of the avermectins for TB therapy. Thus, the avermectins might be readily adopted into the limited repertoire of drugs available to treat MDR and XDR TB. However, *in vivo* pharmacokinetic and efficacy studies will be needed to assess their clinical application for treating TB.

The apparent specificity of avermectins for mycobacteria implies that their target is unique to this taxon of bacteria. The remarkable complexity and uniqueness of the mycobacterial cell envelope, not present in other bacteria that are resistant to avermectins (including *Streptomyces lividans* and *Rhodococcus jostii*, both related actinobacteria), suggest this structure as a potential target (26). In invertebrate nematodes, avermectins specifically bind to glutamate-gated chloride channels present in nerve and muscle cells, causing paralysis and reduced ability to reproduce (27). Avermectins are orally active and distributed to most parts of the human body, including the lungs (24, 28). Ivermectin does not bind ligand-gated chloride channels in mammals, possibly due to impermeability of the blood-brain barrier and nonspecific pumps that prevent it from reaching the central nervous system (20, 27). Interestingly, moxidectin and selamectin, compounds used extensively in animals to treat a variety of parasitic indications without reported toxicity issues, were also highly active against *M. tuberculosis*. The specificity of avermectins for mycobacteria would be ideal for selectively targeting the pathogen while minimizing deleterious effects on resident gut flora after oral administration.

In summary, this is the first report demonstrating the antimycobacterial activity of avermectins. Their established safety profiles in humans and animals make them potential therapeutic options for treating TB. Future work will be done to identify the mycobacterial target of avermectins, to characterize pharmacokinetic and pharmacodynamic properties in animal models using dosages that are higher than that traditionally needed to treat river blindness, and to define the synergistic drug profiles for possible applications in combinatorial TB therapies.

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