LspA-independent action of globomycin on *Mycobacterium tuberculosis*

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**Objectives:** The objective of this study was to investigate the antimicrobial activity and specificity of globomycin, an inhibitor of lipoprotein signal peptidase II (LspA), against *Mycobacterium tuberculosis*.

**Methods:** The mycobactericidal and mycobacteriostatic activity of globomycin was determined by optical density and cfu plating. The specificity of globomycin was determined by western immunoblotting using anti-MPT83 antibody.

**Results:** Globomycin is mycobactericidal at concentrations ≥40 mg/L. However, at 80 mg/L, the processing of the lipoprotein MPT-83 is unaffected and growth-inhibitory effect of globomycin is unchanged in an *lspA* null mutant (Δ*lspA::lspAmut*) lacking the putative drug target.

**Conclusions:** Globomycin kills *M. tuberculosis* through a mechanism that is independent of LspA.

Keywords: lipoproteins, lipoprotein signal peptidase, lipoprotein processing, antimycobacterials

**Introduction**

*Mycobacterium tuberculosis* is among the leading infectious causes of mortality worldwide with 2 million estimated deaths each year.1 One reason why *M. tuberculosis* is a serious threat to tuberculosis control programmes is the global prevalence of drug-resistant and multidrug-resistant strains.2 Drug resistance is a known risk factor for treatment failure, transmission and death.2,3 As we shift towards individualized therapies based on the susceptibility profile of each isolate,4 there is a growing need for new anti-tuberculosis drugs with novel mechanisms of action.

Lipoprotein signal peptidase II (LspA) is a bacterial lipoprotein-processing enzyme that cleaves the signal sequence from prolipoproteins after they are secreted and lipid-modified in the cell wall.5 We and others have shown that deletion or disruption of *lspA* in *M. tuberculosis* results in the severe attenuation of *M. tuberculosis* in the mouse lung (N. Banaiee, E. Z. Kincaid, S. G. Lin, E. Desmond, W. R. Jacobs and J. D. Ernst, unpublished results).6 Given the absence of LspA homologues in mammalian cells, the mycobacterial LspA provides an attractive drug target in *M. tuberculosis*. Globomycin is a peptide antibiotic that is made by several *Streptomyces* species and inhibits Gram-negative bacteria through the inhibition of LspA.7 Globomycin derivates have also been shown to have potent activity against Gram-positive bacteria.8 Thus, it has been proposed that globomycin should be exploited for further drug development against *M. tuberculosis*. In this report, we investigated the activity and specificity of globomycin against *M. tuberculosis*. We show that although globomycin kills *M. tuberculosis* at concentrations of ≥40 mg/L, it does so independently of LspA.

**Materials and methods**

**Bacteria**

All *M. tuberculosis* strains were grown in shaking cultures at 80 rpm to mid-log phase in Middlebrook 7H9 broth (Difco) supplemented with 0.2% glycerol, 10% OADC and 0.05% Tween 80. *Escherichia coli* was grown in shaking Luria broth (Difco) cultures.

**Antibiotics**

Globomycin was kindly provided by Sankyo Co. (Tokyo, Japan) and dissolved in ethanol and used at 10 mg/mL.
Bacterial cultures were grown to mid-log phase and diluted in the respective media to an optical density of 0.05 at a wavelength of 600 nm. Globomycin was subsequently added at the indicated concentrations. An equal volume of solvent was used to achieve each drug concentration. At indicated time points, 200 μL of live culture was aseptically removed in duplicate and the optical densities were measured using a SpectraMax 340PC 384 spectrophotometer. When indicated, cultures were also enumerated by serial dilution and plating on Middlebrook 7H9/ADC agar.

**Western blotting**

To extract mycobacterial proteins, 1 mL of bacterial culture was sedimented and washed once with 1 mL of PBS and resuspended in 300 μL of extraction buffer (50 mM Tris–HCl pH 7.5, 5 mM EDTA, 0.6% SDS, 10 mM NaPO₄ and Roche Complete Protease Inhibitor Cocktail). The suspension was added to 0.1 mL of 0.1 mm zirconia/silica beads and the tube was vortexed for 5 min and subsequently sedimented for 2 min at 12 600g. The supernatant was removed and the protein concentration was determined with the BCA protein assay kit (Pierce). A total of 0.3–1.5 μg of protein was separated on a 12% SDS–PAGE gel and immunoblotting was performed as described previously. Polyclonal anti-MPT83 was kindly provided by Dr Harald Gotten Wiker (Gades Institutt, Norway) and incubated overnight at 4°C at a dilution of 1:2000.

**Results and discussion**

**Globomycin is mycobactericidal**

Globomycin non-competitively inhibits LspA in *E. coli*, which ultimately results in bacterial killing. To determine the activity of globomycin against *M. tuberculosis*, we measured the
bacterial growth rate with a spectrophotometer at various concentrations of globomycin. As shown in Figure 1(a), globomycin was found to inhibit bacterial replication at ≥40 mg/L with complete inhibition occurring at 160 mg/L. When treated bacteria were diluted and enumerated on agar, an approximately 50-fold reduction in viability was observed at 80 mg/L, whereas complete killing occurred at 160 mg/L. Although the bactericidal concentration of globomycin was high in M. tuberculosis, we found that in top 10 E. coli, globomycin was active at 20 mg/L (Figure 1b).

Mycobactericidal activity of globomycin is LspA-independent

The finding that globomycin kills M. tuberculosis is surprising because mutation of lspA is not lethal in M. tuberculosis.\(^6,9\) To test the hypothesis that the mycobactericidal activity of globomycin is independent of its effect on LspA, we evaluated lipoprotein processing in M. tuberculosis after treatment with globomycin. As shown in Figure 2, compared with wild-type and lspA mutant, the signal sequence peptidase activity of M. tuberculosis was unaffected after 6 days of treatment with globomycin at 80 mg/L. Furthermore, to determine whether the activity of globomycin on M. tuberculosis is independent of LspA, we evaluated the replication of M. tuberculosis lspA mutant,\(^9\) which lacks the putative drug target, in the presence and absence of globomycin. If the activity of globomycin on M. tuberculosis were due to specific inhibition of LspA, then we would not expect to see any growth inhibition in the lspA mutant. As shown in Figure 3, treatment of M. tuberculosis lspA mutant with globomycin resulted in killing, similar to that seen in the wild-type bacteria. Together, these findings unambiguously confirm that globomycin does not inhibit LspA in M. tuberculosis and that the mycobactericidal activity of globomycin against M. tuberculosis is independent of LspA.

Given that LspA is essential for the full virulence of M. tuberculosis in mice,\(^6\) it has been suggested that globomycin should be exploited for further drug development against M. tuberculosis. However, the results presented here show that globomycin lacks any LspA-dependent activity against M. tuberculosis and thus it would not be useful as an inhibitor of LspA in M. tuberculosis. Although globomycin is known to inhibit LspA in Gram-negative and Gram-positive bacteria, its LspA-independent toxicity in these bacteria has not been determined.

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Transparency declarations

None to declare.

References