Binding and Action of CEM-101, a New Macrolide/Ketolide in Development for Treating Infections with Macrolide-Resistant and Macrolide-Susceptible Bacteria

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Background:

Resistance has limited the use of macrolides for treating infections. CEM-101 is a new macrolide that is active against macrolide resistant bacteria. The drug-binding site in the ribosome and the action of a novel ketolide, CEM-101, and a related 3-cladinose-containing macrolide, CEM-103, were characterized in this study.

Methods:

The binding of CEM-101 to *E. coli* and *S. aureus* ribosomes was studied by competition binding assays. The inhibitory activity of CEM-101 on protein synthesis was investigated in bacterial and mammalian cell free systems. The binding site of CEM-101 and CEM-103 in the ribosome was characterized by RNA footprinting.

Results:

CEM-101 displayed a tight binding to the ribosome from sensitive Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria with dissociation constants of 66 nM and 47 nM, respectively. CEM-101 inhibited bacterial cell-free translation system with an IC_{50} of 1.1 μM, and showed high selectivity for bacterial vs. mammalian ribosomes. In their binding site in the large subunit of *E. coli* and *S. aureus* ribosomes CEM-101 and CEM-103 establish interaction with the nucleotide residues of the central loop of domain V. In addition, both compounds protect A752 in the loop of the helix 35 in domain II of 23 S rRNA. In the footprinting experiments carried out with the ribosomes isolated from the *erm*-positive *S. aureus* strain N315, both CEM-101 and CEM-103 exhibited characteristic protections of nucleotide residues A752, A2059, G2505 and U2609. This result argues that both compounds are able to bind to the ribosomes dimethylated at A2058 by the action of *erm* methyltransferase.

Conclusions:

The new macrolides CEM-101 and CEM-103 demonstrate high and selective inhibitory activity in the *in vitro* protein synthesis assay. Both compounds bind in the characteristic macrolide-binding site in the large ribosomal subunit. However, CEM-101 and CEM-103 appear to exhibit superior ability to bind to the ribosomes dimethylated at 2058.