Background

The drug-binding site in the ribosome and the action of a novel ketolide, CEM-101, and a related 3-cladinose-containing macrocid, 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 IC50 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 helix 35 in domain II of 23S rRNA.

CEM-101 and CEM-103 show considerable binding to the ribosome arm-dimethylated at A2058, ribosomes were isolated from the arm-positive S. aureus strain N315 and interaction of the drugs with the ribosome was assessed by RNA probing.

Affinity for the Ribosome

The affinity of CEM-101 for E. coli and S. aureus ribosomes was studied by competition binding with [14C] erythromycin, using size-exclusion chromatography. The binding site of CEM-101 and CEM-103 in the ribosome of E. coli, S. aureus, and S. aureus strain N315 was characterized by RNA footprinting with DMS, kethoxal, and CMCT.

CEM-101 competes for the ribosome-binding site with erythromycin with an IC50 of 155 nM for the E. coli ribosome, and 117 nM for the S. aureus ribosome.

Interaction of CEM-101 with the wild-type ribosome

Interaction of CEM-101 with the E. coli and S. aureus ribosomes was interrogated by RNA probing.

Interaction of CEM-101 with the m5A2058 ribosome

In order to test the ability of CEM-101 and CEM-103 to bind to the ribosome arm-dimethylated at A2058, ribosomes were isolated from the arm-positive S. aureus strain N315 and interaction of the drugs with the ribosome was assessed by RNA probing.

The binding site of CEM-101 and CEM-103 show considerable binding to the ribosome arm-dimethylated at A2058. Judging by the efficiency of protection, CEM-101 and CEM-103 have a higher affinity to the arm-modified ribosome than telithromycin, azithromycin or erythromycin.

Inhibition of Protein Synthesis

The inhibitory activity of CEM-101 on protein synthesis was investigated in E. coli cell-free transcription-translation assay and in eukaryotic (rabbit reticulocyte lysate) cell-free translation system. CEM-101 inhibited the synthesis of firefly luciferase in an E. coli cell-free transcription-translation assay with an IC50 of 1.1 μM.

Induction of ermC

The ermC inducing activity of CEM-101 and CEM-103 was analyzed using pERM30 reporter in the E. coli JM109 cells. The ability of CEM-101 and other macrolides to induce the stalling of the ribosome at the ermC ORF was tested by toe-printing assay in vitro.

Conclusions

CEM-101 binds with a high affinity to the bacterial ribosome and demonstrated high and selective inhibitory activity in the in vitro protein synthesis assay. CEM-101 and CEM-103 bind in the characteristic macrolide-binding site in the large ribosomal subunit. Due to the presence of alkyl-aryl side chain they establish interactions with A752 in domain II of 23S rRNA. CEM-101 and CEM-103 appear to exhibit superior ability to bind to the ribosomes arm-dimethylated at A2058 of 23S rRNA. Similar to other ketolides, CEM-101 can induce expression of ermC-based reporter; however, it does not induce the ribosome stalling at the ermC regulatory ORF.

Acknowledgements

This work was supported by the research grant from CEMPRAPharmaceuticals.

Abstract

Affinity for the Ribosome

The affinity for the ribosome of CEM-101 was studied by competition binding with [14C] erythromycin, using size-exclusion chromatography. The binding site of CEM-101 and CEM-103 in the ribosome of E. coli, S. aureus, and S. aureus strain N315 was characterized by RNA footprinting with DMS, kethoxal, and CMCT.

CEM-101 competes for the ribosome-binding site with erythromycin with an IC50 of 155 nM for the E. coli ribosome, and 117 nM for the S. aureus ribosome.

Interaction with the wild-type ribosome

Interaction of CEM-101 with the E. coli and S. aureus ribosomes was interrogated by RNA probing.

Interaction with the m5A2058 ribosome

In order to test the ability of CEM-101 and CEM-103 to bind to the ribosome arm-dimethylated at A2058, ribosomes were isolated from the arm-positive S. aureus strain N315 and interaction of the drugs with the ribosome was assessed by RNA probing.

The binding site of CEM-101 and CEM-103 show considerable binding to the ribosome arm-dimethylated at A2058. Judging by the efficiency of protection, CEM-101 and CEM-103 have a higher affinity to the arm-modified ribosome than telithromycin, azithromycin or erythromycin.

Inhibition of Protein Synthesis

The inhibitory activity of CEM-101 on protein synthesis was investigated in E. coli cell-free transcription-translation assay and in eukaryotic (rabbit reticulocyte lysate) cell-free translation system. CEM-101 inhibited the synthesis of firefly luciferase in an E. coli cell-free transcription-translation assay with an IC50 of 1.1 μM.

Induction of ermC

The ermC inducing activity of CEM-101 and CEM-103 was analyzed using pERM30 reporter in the E. coli JM109 cells. The ability of CEM-101 and other macrolides to induce the stalling of the ribosome at the ermC ORF was tested by toe-printing assay in vitro.

Conclusions

CEM-101 binds with a high affinity to the bacterial ribosome and demonstrated high and selective inhibitory activity in the in vitro protein synthesis assay. CEM-101 and CEM-103 bind in the characteristic macrolide-binding site in the large ribosomal subunit. Due to the presence of alkyl-aryl side chain they establish interactions with A752 in domain II of 23S rRNA. CEM-101 and CEM-103 appear to exhibit superior ability to bind to the ribosomes arm-dimethylated at A2058 of 23S rRNA. Similar to other ketolides, CEM-101 can induce expression of ermC-based reporter; however, it does not induce the ribosome stalling at the ermC regulatory ORF.

Acknowledgements

This work was supported by the research grant from CEMPRAPharmaceuticals.