The C16G2 Mechanism of Action Depends on Selective Interaction With S. mutans Cell Wall and Lipid Membrane Components

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Abstract

C16G2 is a Specifically Targeted Antiglycoprotein Peptide (STAMP) designed to treat the cariogenic pathogen Streptococcus mutans. The C16 peptide region within C16G2 is a derivative of the S. mutans Competence-Stimulating Peptide (CSP), which interacts with and regulates the membrane-bound ComD receptor. Previously it was established that unlike C16, C16G2 does not activate ComD and is equally as potent against S. mutans as wild type S. mutans (Lee et al., 2015). In this study, we further investigated the contribution that cell wall and membrane composition plays in the selectivity of C16G2 for S. mutans. An enantiomer of C16G2 displayed a potency and selectivity profile identical to the L-form, suggesting that peptide activity does not appear to depend on a protein target. Additionally, bacterial strains that are insensitive to C16G2 could be rendered susceptible by removal of teichoic acid (TA), positively-charged choline, or D-AlaD from their cell wall. This suggests that a component of selectivity of C16G2 for S. mutans may be due to lack of choline and/or lower D-AlaD-substituted TA on its cell wall, as compared to levels in other bacteria.

C16G2 Enantiomer Activity and Selectivity

A C-16G2 D-form peptide was created to test the effects of chirality on binding with specific proteins. The activity and selectivity of the C16G2 enantiomers were tested against Streptococcus mutans by a time-kill assay against planktonic bacteria and a mixed culture assay of S. mutans versus total streptococci, respectively. The activity and selectivity of C16G2 was not altered by changing the chirality.

Bacterial cell wall composition effect on C16G2 selectivity

Sodium periodate (NaO4) breaks down wall teichoic acids (WTA) and lipoteichoic acids (LTA) on bacterial cell wall. A NaO4 treated (red line) and untreated (blue line) S. mutans were treated with 0.25 μM C16G2.

The net charge of the bacterial cell wall is a factor in determining C16G2 potency and selectivity

Lipoteichoic acid (LTA) and wall teichoic acid (WTA) are two cell wall components targeted by sodium periodate and impartarily structural alterations in LTA and WTA exist between different bacteria. Therefore, we investigated the interaction of C16G2 with S. mutans LTA and the potential role these alterations may have on its activity.

C16G2 interacts with LTA. Exogenous LTA was treated with periodate to prevent the LTA from forming micelles and potentially trapping peptides. The activity of a C16G2/LTA mixture at 0.1% concentration ratio (μg/L) was tested against S. mutans through a time-kill assay. The C16G2/LTA mix was inactive against S. mutans. This suggests C16G2 is saturated by excess LTA.

Conclusions

The activity and selectivity of C16G2 is not altered by changing peptide chirality, indicating that the selectivity of C16G2 for S. mutans does not likely depend on a protein target.

References


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