The effects of sub-inhibitory concentrations of a PolC-inhibitor on *Clostridium difficile*

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PolC inhibitors like 362E act as a competitive dGTP analog

The DNA-polymerase (PolC) of Gram positive organisms is an attractive target for the development of novel antimicrobials as it is essential for replication and absent in Gram negative bacteria. 362E (N^2-(3,4-Dichlorobenzyl)-7-(2-[1-morpholinyl]ethyl)guanine; MorE-DCBG) is a DNA polymerase inhibitor in pre-clinical development as a novel therapeutic against *C. difficile* [1].

Template-directed elongation is blocked by the inhibitor through simultaneous binding to the cytosine of the DNA strand and near the active site of PolC **(Figure 1 A-B**).This synthetic purine shows preferential activity against *C. difficile* PolC over those of other organisms *in vitro* and is effective in an animal model of *C. difficile* infection [2].

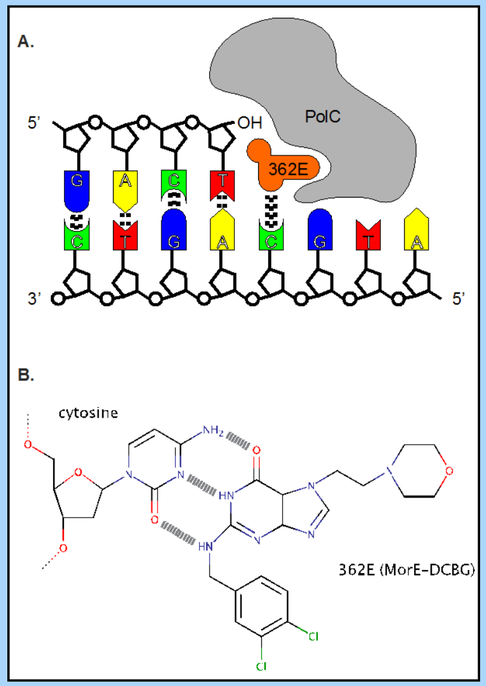

Figure 1. (A) Ternary complex of inhibitor 362E, DNA ,and PolC. (B) H-bonding between inhibitor molecule 362E and a cytosine residue of DNA.

362E is a potent inhibitor of diverse clinical isolates of *C. difficile*

DNA polymerase inhibitors HPUra and 362E were tested by agar dilution method, according CLSI guidelines, against 363 *C. difficile* clinical isolates collected earlier in the framework of a pan-European study [3]. All strains were characterized by PCR-ribotyping and presence of genes encoding toxins A, B and binary toxin. 362E (MIC_{50}: 2 µg/ml; MIC_{90}: 4 µg/ml) demonstrates lower inhibitory concentrations than the general Gram-positive PolC inhibitor HPUra (MIC_{50}: 16 µg/ml; MIC_{90}: 32 µg/ml) (**Figure 2A),** consistent with previous in vitro activities against purified PolC [1].

Furthermore, we have confirmed that *B. fragilis*, devoid of PolC, was resistant to both polymerase inhibitors (MIC >265 µg/ml). No differences in 362E susceptibility was observed between clades (data not shown). Type 014 seemed to be less sensitive to 362E, compared to the other clinical relevant PCR-ribotypes (**Figure 2B**).

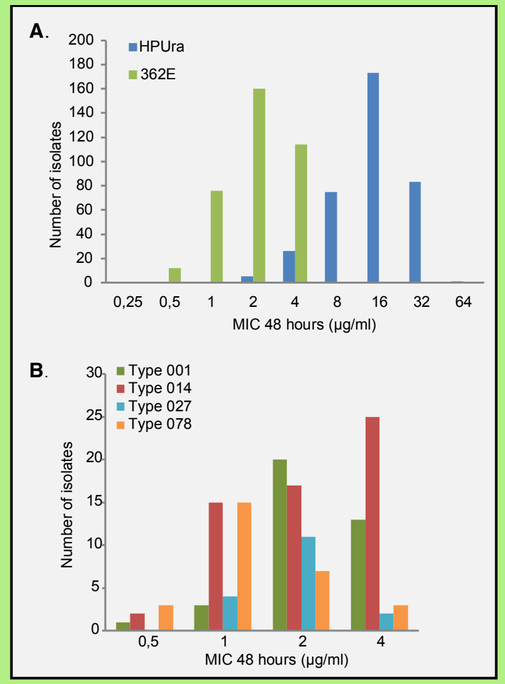

Figure 2. Minimum Inhibitory Concentrations determined by agar dilution method (A): MIC distribution inhibitors (n=363). (B): MIC distribution 362E of clinical relevant PCR-ribotypes.

Growth kinetics are severely affected at sub-MIC levels of 362E

C. difficile 630*Δerm* was grown in BHI/YE with appropriate amounts of 362E, starting from an OD_{600} of 0.05 using an exponentially growing starter culture. The effect on growth was most prominent five hours post inoculation (**Figure 3**).

Growth kinetics of cultures containing inhibitor ≥ 1 µg/ml were similar and resulted in 30 percent less growth compared to the untreated culture.

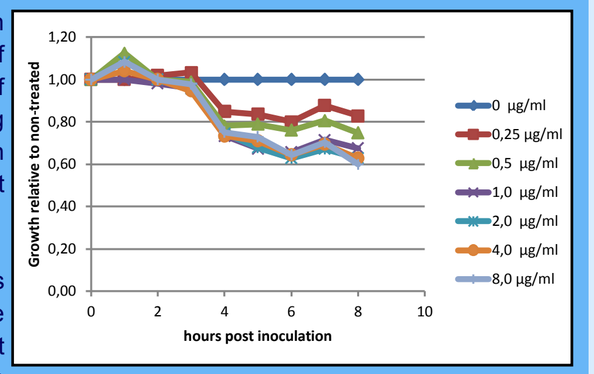

Figure 3. Relative growth kinetics of *C. difficile* 630*Δerm* cells under various concentrations of 362E vs. non-treated cells

Gene dosage shift occurs under sub-inhibitory concentration of 362E PolC-inhibitor

Clostridium difficile has a single circular chromosome and one origin of replication (*oriC*) from which the process of replication occurs bi-directionally (**Figure 4A**). Marker frequency analysis (MFA) can be performed to determine the relative abundance of origin proximal genes relative to terminus (*terC*) proximal genes. We found that *C. difficile* demonstrates multi-fork replication in exponential growth phase and that MFA also detects the expected decrease in *oriC*:*terC* ratio when cells enter stationary growth phase (data not shown). When cells were treated with 362E (4 µg/ml), an 8-16-fold increased *oriC*:*terC* ratio was observed, which was absent from cells treated with chloramphenicol (2 µg/ml), a protein synthesis inhibitor (**Figure 4B**).

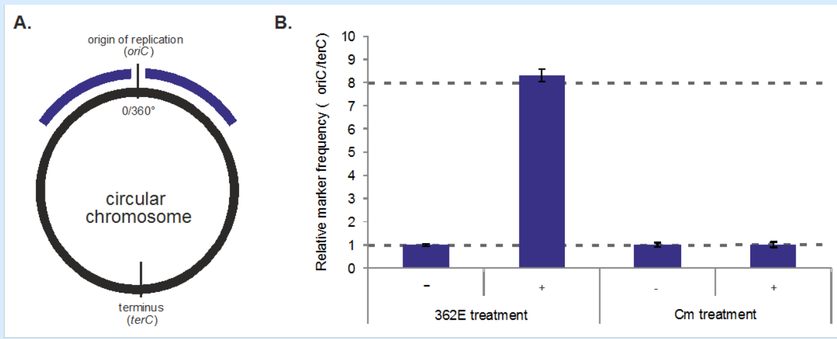

Figure 4. (A) Bi-directional replication of prokaryotes. (B) Marker Frequency Analysis of sub-inhibitory effects of PolC inhibitor 362E compared to the antibiotic Chloramphenicol (Cm).

Work in the group of W.K. Smits is supported by a Gisela Thier Fellowship (LUMC)

References

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