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LTX-109 Demonstrates Rapid Membranolytic Activity against *Escherichia coli* and *Staphylococcus aureus*

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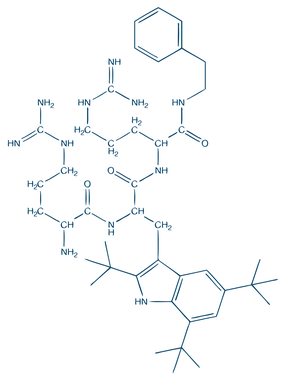
Lytix Biopharma

LTX-109

- A synthetic protein fragment - a peptidomimetic
- High stability against degradation
- Prooduced synthetically in large scale
- Low cost of synthesis



Chemical structure of LTX-109.

Background

LTX-109 is a novel antimicrobial drug in clinical development for skin infections and nasal decolonisation of MRSA. The drug mimics the effects of natural antimicrobial peptides in a synthetic small molecule. LTX-109 has demonstrated a broad activity against several Gram (+) and Gram (-) bacteria *in vitro*, as well as activity against a range of yeast and fungal species. The compound is equally effective against antibiotic-resistant species such as methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococci* (VRE) and multi-resistant *Pseudomonas* isolates. The ultra-rapid membrane lysing mode of action may result in a lower propensity to resistant development and a rapid bactericidal mechanism of action, as shown in *in vitro* studies. To date LTX-109 demonstrates no *in vitro* cross-resistance with other classes of antibiotics.

The present study investigated the mode of action of LTX-109 by means of bacterial real-time assays.

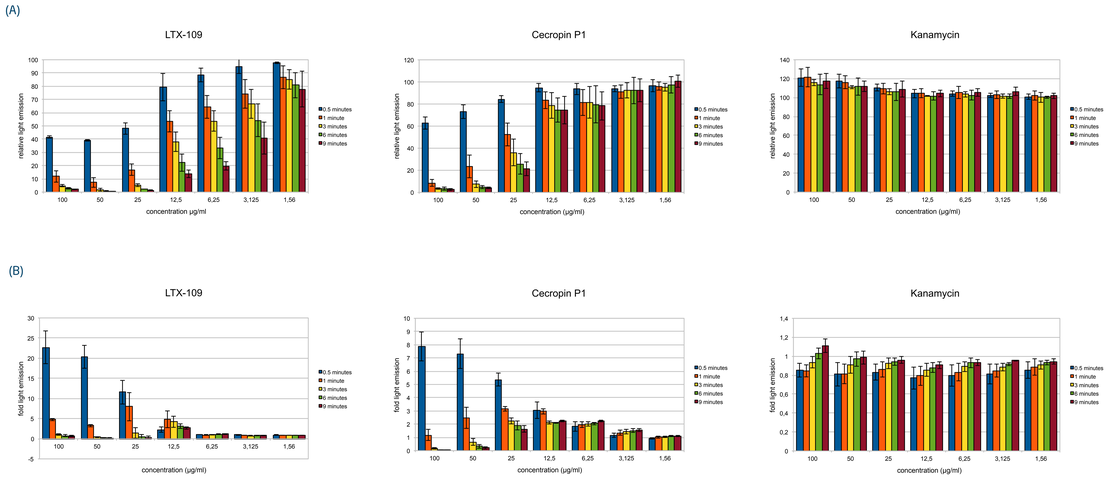
Methods

A real-time viability assay based on the expression of bacterial luciferase in *E. coli* and *S. aureus* was used to assess the metabolic state of sensor bacteria. Light produced by *E. coli* HB101 constitutively expressing the *luxCDABE* operon indicates viable bacterial cells. Reduced relative light emission indicates bactericidal activity.

A second real-time assay based on *E. coli* cells expressing insect luciferase was used to analyse bacterial cell membrane integrity. Relative light production by *E. coli* HB101 constitutively expressing an insect luciferase requires externally added D-luciferin as its substrate. Light induction results from the increased passage of D-luciferin into the bacteria in response to decreased membrane integrity induced by the treatment with AMPs. Concentrations of LTX-109 from sub-MIC to 5xMIC were used for both assays.

Results

LTX-109 demonstrated rapid membranolytic activity against *E. coli* (see figure below) and *S. aureus* (not shown) in the real-time Luciferase cell viability assay. In *E. coli* this quick response was comparable to results obtained with cecropin P1, which is a well-known membrane active antimicrobial peptide. In the membrane-integrity assay, at MIC and higher concentrations LTX-109 induced a peak of light emission comparable to cecropin P1, whereas at sub-MIC-concentrations of LTX-109 no light peak was observed.



Dose and time dependent effect of LTX-109, Cecropin P1 and Kanamycin on *E. coli* viability (A) and membrane integrity (B). Changes in light production in response to treatment with different concentrations of the respective antimicrobial agents are shown relative to the untreated control for the 4 selected incubation times. The mean of three independent measurements is indicated for 0.5, 1, 3, 6 and 9 minutes after addition of the peptides.

Conclusions

- LTX-109 appears to kill bacteria by interfering with plasma membrane integrity.
- Since this activity is measurable at concentrations equal to the MIC, we propose that the main mode of action is damaging the bacterial plasma membrane.
- The drug has been tested in Phase I and two Phase I/IIa trials with good tolerance, minimal systemic bioavailability
- LTX-109 has demonstrated Proof-of-Concept in decolonisation of nasal MRSA / MSSA.
- Further Phase II studies are planned to demonstrate efficacy in larger patient populations.

LTX-109

- Novel mechanism of action
- Broad spectrum of activity
- Low propensity for resistance development and active against drug-resistant strains
- Effective against fungal and bacterial biofilms
- Superior efficacy compared to market leaders (Bactroban®, Fucidin®, Altabax®/Altargo®)

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