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Paper Poster Session
Microbial pathogenesis and virulence

D-amino acids as anti-virulent agents in Gram-negative pathogens as Pseudomonas aeruginosa and Acinetobacter baumannii

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Background: Biofilm formation is an important requirement for chronic colonization of human tissues, persistence in implanted medical devices and it is associated with multidrug resistance. On the other hand, bacterial adherence to eukaryotic cells is a crucial step in the infection process. D-aminoacids are produced by bacteria in stationary phase and play a potential role as modulators of bacterial growth and persistence. Their ability to inhibit biofilms is a very controversial issue.

We have analyzed the effects of 18 D-aminoacids on *A. baumannii* and *P. aeruginosa*. *In vitro* assays were developed to analyze their effect on growth, biofilm formation/disassembling and capacity to adhere to eukaryotic cells. Also *in vivo* assays were performed to investigate the D-aminoacids potential for the treatment of sepsis and pneumonia infections in mice.

Material/methods: *A. baumannii* ATCC17978 and *P. aeruginosa* PAO1 were grown in *polystyrene* 96-well plates for 48 h using M9 and M63 medium, respectively, supplemented with different D-aminoacids. Biofilm formation was measured using crystal violet staining. *MICs and growth curves were carried out.*

Cell adhesion assays were performed using A549 human cells cultured *in* 24-well plates. The monolayer was infected with *A. baumannii* or *P. aeruginosa* in modified Hank's balanced salt solution supplemented with different D-aminoacids for 3 h.

Experimental murine pneumonia and sepsis models were used to evaluate the efficacies of D-aminoacids against the *A. baumannii* and *P. aeruginosa* strains. Pharmacokinetics (PK) and Pharmacodynamics (PD) parameters of D-aminoacids were assessed by HPLC.

Results: Biofilm formation in *A. baumannii* was inhibited in the presence of D-His, D-Cys and D-Trp (20-50% and 45-95% of inhibition at 0,5 mM and 4 mM, respectively) and *D-Cys, D-Trp and D-Tyr* (less than 10% at 0,5 mM and 32-19% at 6 mM) in *P aeruginosa*.

Adherence experiments showed that the presence of D-Cys, D-His and D-Met at 1mM, and D-Arg and D-Trp at 1mM significantly reduced the ability of *A. baumannii* and *P. aeruginosa* to attach to eukaryotic cells, respectively.

MICs and growth curves revealed that only D-Cys at 1 mM was toxic for *A. baumannii* and D-Trp at 8 mM in *P. aeruginosa.*

Pk/PD parameters showed that effective dosis of D-aminoacids (1 mM) requires a PK/PD model with high doses of D-aminoacids, which produce toxic effects. Murine infections revealed that the best survival rates was the treatment 300 mg/Kg of D-His intraperitoneally each 4 h (p=0.09) in *A. baumannii* sepsis model and the treatment 300 mg/kg of DCys-DTrp intraperitoneally each 4 h (p=0.2) in pneumonia model *P. aeruginosa.*

Conclusions: D-aminoacids can inhibit growth, biofilm formation and adherence to eukaryotic cells abilities of *A. baumannii* and *P. aeruginosa*. Although, treatments with D-aminoacids in murine infections have shown differences in clinical response, there are no significant differences in survival rates.