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## UNVEILING THE POSSIBLE MECHANISM OF ACTION AGAINST GRAM NEGATIVE BACTERIA AND INTERACTION BETWEEN CANNABIDIOL (CBD) AND COLISTIN

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In recent years, resistance to colistin (*col-R*), the last line of defense in clinical treatment against resistant bacteria, has disseminated among various Gram-negative bacteria such as *Escherichia*, *Salmonella* and *Klebsiella*. The non-psychoactive cannabinoid CBD is a lipophilic molecule that displays antimicrobial activity, mainly against Gram-positive bacteria. However, in Gram negatives it does not show any antibacterial activity. This phenomenon it is supposed to be a result of the external membrane that would act as a barrier for this molecule. In previous works we demonstrated the synergy between CBD and Colistin against various Col-R gram-negative strains. But the actual mechanism of action during this synergy still remains uncertain. In this work we addressed different assays in an attempt to unveil the possible mechanism of action or interaction between these two compounds when acting together on Gram negative bacteria. First, we evaluated the interaction between these two antimicrobials using NMR and we observed that these two molecules interact with each other in a stoichiometric relationship of 1:1. We could observe that there is equilibrium where exchange is rapid (on the microsecond scale). In principle, at the 1:1 equivalent condition, all CBD and colistin molecules are interacting with a regime of rapid exchange. This result prompted us to question if this interaction was necessary before contact with bacterial membrane for the antimicrobial action to take place. To address this question microbiological assays were performed. In the first assay, CBD and colistin were incubated in culture medium for 30 minutes before being added to the plate with the bacteria. In the second assay, one antibiotic was incubated with the bacteria and after 30 minutes, the other antimicrobial was added. As a control the two treatments were added simultaneously together with bacteria. The plate was incubated for 24 hs and the bacterial growth was evaluated. The

results showed that when colistin was incubated with bacteria and CBD was added 30 minutes later, the synergistic effect was seriously diminished, requiring eight-fold more CBD to reach synergy. Scanning electron microscopy was also performed in order to obtain images of the bacterial membrane after incubation with CBD, colistin or the combination. In these images we could observe that CBD alone did not affect the membrane at all, but colistin at sub-MIC concentration produced blisters or bubbles on the surface of the bacteria external membrane. When both compounds were included together, the membrane lysis was clearly observed. All together the results showed that CBD alone do not affect the bacterial membrane, but its interaction with colistin drives a synergistic antibiotic action on Gram negative bacteria through possible membrane disruption.

Palabras clave: Cannabidiol – colistin – synergy - E. coli