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P2807 Mechanism of the bactericidal action of combined tomatidine and aminoglycoside against *Staphylococcus aureus* virulent and persistent phenotypes

Jean-Philippe Langlois*[1], Isabelle Guay[1], Viktor Steimle[1], Kamal Bouarab[1], Eric Marsault[2], François Malouin[1]

[1] Faculte des sciences, Departement de biologie, Université De Sherbrooke, Sherbrooke, Canada, [2] Faculte de medecine et des sciences de la sante, Departement de pharmacologie et de physiologie, Université De Sherbrooke, Sherbrooke, Canada

Background: *Staphylococcus aureus* (SA) can adopt two phenotypes, prototypical (WT) and its small-colony variant (SCV). Tomatidine (TO), a steroidal alkaloid, exhibits a bactericidal effect on SCV. Moreover, when TO is mixed with an aminoglycoside such as gentamicin (GEN), the combination shows a strong synergistic activity against WT. We recently determined that the molecular target of TO was the ATP synthase subunit c (AtpE) and we report here how TO, with or without GEN, exerts its bactericidal activity against WT and SCV.

Materials/methods: Since TO affects the bacterial ATP synthase, we measured the membrane potential. Bacteria in broth were incubated with various concentrations of antibiotics (TO or TO-GEN). Bacteria were then washed in PBS and the fluorophore $DiOC_2$ was added and incubated for 30 min before flow cytometry. To assess the production of reactive oxygen species (ROS), bacteria were suspended in broth and incubated 2h at 35°C before the addition of 10 µM H_2DCFDA for 1h. Bacteria were then washed and transferred to a 96-well plate containing broth and antibiotics (ciprofloxacin as a control). Fluorescence was measured (λ exc 494_{nm}, λ emi 521_{nm}) over a 13-h period. All results are reported as a percentage of that measured for WT without antibiotic.

Results: TO reduced WT membrane potential in a dose-dependent manner and reached a low of 35% at the highest doses (≥8 µg/mL). On the other hand, SCV membrane potential, which was about 10% of that of WT, further dropped to about ≤2% at very low TO concentrations (≥0.0035 µg/mL). This was also accompanied by 2 times more ROS than that seen in the no antibiotic control. Besides, there was no difference in membrane potential of WT when comparing the effect of GEN to that of TO-GEN. However, the combination TO-GEN generated 2.5 times more ROS compared to that caused by GEN alone.

Conclusions: TO is able to reduce membrane potential of both WT and SCV, but significant ROS are only produced in SCV, which are highly susceptible to TO. Similarly, only the TO-GEN combination generated significant ROS production in the WT, which explains the strong synergy with aminoglycosides.