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**In vitro effect of hypoxia on the infections caused by *Acinetobacter baumannii* and *Pseudomonas aeruginosa***

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**Background:** Hypoxia is known to modulate the bacterial virulence through the hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), a key regulator which activates gene expression in response to hypoxemic or inflammatory conditions, allowing immune cells to function better under low oxygen concentration. The objectives of this study were to analyze the *in vitro* effect of hypoxia on the infections of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* and therefore to characterize the innate immune response *in vitro*.

**Material/methods:** HIF-1 $\alpha$  levels have been measured in human lungs epithelial cells (A549 cells) and murine macrophages (RAW cells) after 6 and 24h under hypoxia (1% of oxygen) and normoxia (21% of oxygen) by ELISA. To evaluate the effect of hypoxia on the bactericidal activity and on the bacterial adherence, both cells have been infected with *A. baumannii* ATCC 17978 and *P. aeruginosa* PAO1 strains with a multiplicity of infection of 500. Bacterial concentrations in the extracellular medium and adhered to both cells have been measured 2 and 24h post-infection under hypoxia and normoxia. To characterize the *in vitro* innate immune response in these bacterial infections, levels of IL-6, IL-10 and TNF- $\alpha$  in the extracellular medium have been measured by ELISA.

**Results:** In A549 and RAW cells, the highest HIF-1 $\alpha$  levels were reached after 6h in hypoxia (2296.98 $\pm$  273.33 and 331.64 $\pm$ 91.68pg/ml, respectively). Subsequently, in the hypoxia experiments, the infection was always carried out after 6h of hypoxia cell cultures. Incubation of A549 cells under hypoxia, for 2 and 24h with ATCC 17978 and PAO1, increased their bactericidal activity by reducing the bacterial concentration in the extracellular medium to 50.49 $\pm$ 13.01% and 42.46 $\pm$ 14.52% for ATCC 17978, and 90.84 $\pm$ 24.09% and 60.10 $\pm$ 20.05% for PAO1,

respectively. Incubation of RAW cells under hypoxia, for 2 and 24h with ATCC 17978 and PAO1, increased their bactericidal activity by reducing the bacterial concentration in the extracellular medium to  $67.58 \pm 31.50\%$  and  $29.44 \pm 0.30\%$  for ATCC 17978, and  $50.26 \pm 18.88\%$  at 24h for PAO1. On the other hand, the bacterial adherence of both strains to both cells was lower in hypoxia, except in the case of 2h post-infection by ATCC 17978 in RAW cells which was higher ( $182.67 \pm 19.05\%$ ).

No differences in the innate immune response in the A549 cells infected by both pathogens were found between hypoxia and normoxy. In the case of RAW cells, there were significant changes between hypoxia and normoxy, after 24h post-infection by ATCC 17978 in IL-6 levels and by PAO1 in TNF- $\alpha$  levels.

#### **Conclusions:**

1. Hypoxia increased bactericidal activity of host cells against *A. baumannii* and *P. aeruginosa* and reduced the adherence of these pathogens.
2. Significant differences in the innate immune response in RAW cells, but not in A549 cells, were found between hypoxia and normoxy.