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In vivo effect of hypoxia on infections caused by Acinetobacter baumannii and Pseudomonas aeruginosa in different murine models

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Background: Immunotherapy by enhancing the immune system is a promising alternative in the treatment of gram-negative bacilli. Hypoxia modulates bacterial virulence through the hypoxia inducible factor-1α (HIF-1α), allowing the immune system to function under low oxygen concentration. The objective of this study was to analyse the effect of hypoxia on infections caused by gram-negative bacilli in different animal models, regarding to survival, tissues bacterial concentrations, and cytokine release.

Material/methods: Two murine models of infection, under hypoxia (10% oxygen) and normoxy, were used: a peritonitis sepsis model by *A. baumannii* ATCC 17978 and a pneumonia model by *P. aeruginosa* PAO1. Immunocompetent male mice C57BL/6 were used. The minimum lethal doses (MLD) were calculated under both conditions (Reed and Munch method). For both models, tissues and fluids were extracted, processed and analysed 4h post-inoculation and at time of death. Survival rates were also analysed. The innate immune response was evaluated by measuring IL-6, IL-10, and TNF-α levels (ELISA). HIF-1α levels were measured by ELISA. Statistical analysis: ANOVA (bacterial counts in tissues and fluids and mortality time), Chi-square test (bacteremia) and Student's t-test (cytokines and HIF-1α levels); a $p<0.05$ was considered significant.

Results: Peritonitis sepsis model: MLD (hypoxia and normoxy) were 2.08 and 3.20 $\log_{10}$ cfu/ml, respectively. No differences in bacterial concentration in tissues, peritoneal fluid and blood were found 4h post-infection. After death, approximately 24h, differences in the bacterial concentration (PF, lungs and blood) were found 8.88±0.53 *vs.* 9.31±0.33 cfu/ml, 8.25±0.54 *vs.* 9.36±0.35 cfu/g and 7.73±0.20 *vs.* 8.28±0.58 cfu/ml, respectively. Survival time was longer under normoxy conditions. Higher IL-10

levels 4h post-infection were found in hypoxia *vs.* normoxy, but no differences in IL-6, TNF-α and HIF-1α levels were found.

Pneumonia murine model: MLD (hypoxia and normoxy) were 8.54 $\log_{10}$ cfu/ml. No differences in bacterial concentrations in tissues and blood were found 4h post-infection. Differences in the bacterial concentration (spleen, lungs and blood) were found at the death time: 5.27±0.60 *vs.* 6.96±0.57, 9.04±0.58 *vs.* 9.81±0.45 and 5.66±0.78 *vs.* 7.90±0.67, respectively. Survival time was longer under normoxy conditions. Bacteremia rates after 4h were 61.11% (hypoxia) and 44.44% (normoxy). The IL-6 levels (4h post-infection) and the IL-10 levels (death time) were higher and lower under hypoxia, respectively.

Conclusions: The MLD is different between hypoxia and normoxy in the sepsis model by *A. baumannii*. Survival time was longer under normoxy condition in both models. After death, differences in the bacterial concentration in tissues and fluids were found in the sepsis model by *A. baumannii* and in pneumonia model by *P. aeruginosa*. After 4h infection, IL-10 levels (*A. baumannii*) and IL-6 levels (*P. aeruginosa*) were overexpressed in hypoxia. After death, the levels of IL-10 were lower in hypoxia for the pneumonia model by *P. aeruginosa*.