

BIO_21 - Pharmacological characterization of a specific camelid single-domain antibody and correlated constructs anti-toxins of *Bothrops* venom

Nidiane Dantas Reis Prado¹; Brunheld Maia Dutra¹; Sibele Andrade Roberto²; Nathalya Rocha Duran²; Jackson Vieira Leitão²; Thifany Foschieira de Melo²; Eliza Lima dos Santos²; Carla Freire Celedônio Fernandes¹; Soraya dos Santos Pereira².

¹Fiocruz/Ceará

²Fiocruz/Rondônia

Introduction: The bite of venomous snakes is widely recognized as the primary cause of human harm inflicted by venomous animals globally. The existing constraints associated with currently available antivenoms underscore the necessity for exploring alternative treatments to effectively mitigate the effects of snakebite envenomation. The single-domain antibodies or VHH showing important pharmaceutical characteristics and a high potential to neutralize toxins present in snake venom, can be applied as an adjuvant to available official serotherapy or in pharmaceutical forms.

Objectives: Characterize in vitro the neutralization efficacy of anti-toxin VHHs in a monomeric and multimeric conformation, being a VHH with homodimeric structure against PLA2 (BthTX-I and BthTX-II) and a heterodimeric VHH against PLA2 and BjussuMPII, all toxins from *Bothrops* snake venom.

Methodology: Tests were carried out on murine C2C12 cells differentiated into myotubes, with analysis of the neutralization potential of VHHs against cytotoxicity triggered by *Bothrops jararacussu* venom and the BthTX-I PLA2, through LDH quantification, and analysis cell viability using the MTT method.

Results: In the test with myotubes using a ratio of 1:5 (poison/toxin: VHH), the three conformations of VHH showed a reduction of more than 50% in LDH levels compared to cells that received only venom or toxin. While in the MTT cytotoxicity assay, monomeric and heterodimeric VHH showed cell viability comparable to the negative control in cells incubated with BthTX-I, while the heterodimer showed an increase in viability of approximately 50%, compared to cells that received the total venom, all three conformations of VHH showed an increase in cell viability by more than 50%.

Conclusion: Such data suggest the great potential of VHH and its conformations for therapeutic application against snakebite, demonstrated by the persistence of neutralizing activity by the different conformations used. Further studies to characterize the best format as well as pharmaceutical presentation with greater therapeutic potential are underway and present promising results for the presentation of VHHs as a biopharmaceutical for the treatment of snakebite.

Keywords: VHH; Snakebite; Biopharmaceutical