The *Micrurus lemniscatus* Venom induces L-glutamate Release from Rat Cerebro Cortical Synaptosomes and Shows Toxicity to Cultured Neurons

Donato, M.F.¹; Montandon, A.C.¹; Freitas, A.C.N.²; Ferreira, A.F.²; Sandoval, M.R.L.³; Chaves, M.M.² and De Lima, M.E.².

¹Programa de Pós-Graduação em Fisiologia e Farmacologia, ²Programa de Pós-Graduação em Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG. ³Laboratório de Farmacologia. Instituto Butantan, SP, Brasil.

**Introduction:** Human envenomations by coral snakes result in severe neurotoxicity. The accident may lead to death by respiratory paralysis. The treatment includes specific antivenom produced against *M. corallinus* and *M. Frontalis* venoms. Studies on the central neurotoxic effects of these venoms are scarce. This study proposed to evaluate the toxic effect induced by crude venom (CV) of *M. lemniscatus* on cultured hippocampal neurons and in a Neuro-2A cells challenging with a commercial antivenom. We also investigated the effect of this venom on L-glutamate release in rat brain synaptosomes.

**Material and Methods:** Crude venom (CV) and commercial antivenom were from FUNED (Belo Horizonte, Brazil). Hippocampal neurons culture was prepared from brain of Wistar neonatal rats. Neurons and Neuro-2A cells were treated with 1-10⁻⁵µg CV for 1, 3 and 24h.

Neutralization assays with antivenom were made with 1µg of CV and different concentrations of serum (3-10⁻³µL). Cell survival was quantified by MTT, Neutral red and fluorescence assays. Cerebrocortical synaptosomes were prepared, incubated with CV and L-glutamate release was quantified by a fluorescent assay.

**Results and Discussion:** The CV shows toxicity to hippocampal neurons, in an independent concentration manner. The chronic assay (24h) showed cellular death with 1 and 10⁻²µg CV, and acute assays show toxicity at lower concentrations. CV (10⁻²µg) induced L-glutamate release in synaptosomes in a time-dependent manner. In Neuro-2A cells the CV promoted significant cell death (1-10⁻²µg) and in neutralization tests, the specific antivenom was capable to prevent 50% of the cellular death caused by CV.

**Conclusions:** We conclude that the *M. lemniscatus* CV promoted significant central neurons cell death in a not concentration-dependent manner. We suggest that neurotoxicity could be associated with L-glutamate release. The antivenom was ineffective to completely neutralize the neurotoxic effect of crude venom pointing out a challenge to be solved in envenoming by *Micrurus*.


**Support:** CAPES, CNPq, FAPEMIG, INCTTOX-Fapesp