Cramoll 1,4 and rCramoll 1 have protective effects against hydrogen peroxide induced cell death

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INTRODUCTION: Oxidative stress plays an important role in the induction of cell death and is related with various pathologic disorders. The major source of endogenous reactive oxygen species is H₂O₂ which has been extensively used to induce oxidative stress in vitro models. Isolectins from Cratylia mollis seeds (Cramoll-1, -2, -3, -4) has been described as immunomodulatory, mitogenic, antitumor and healing agent. Recently, the expression of functional recombinant (rCRAMOLL) in Escherichia coli was reported by our group. In this work we evaluated the protective effect of Cramoll 1,4 (pCramoll) and recombinant Cramoll 1 (rCramoll) against H₂O₂-induced dysfunction in Vero cells (monkey fibroblast).

METHODS: The cells were incubated with H₂O₂ (1 mM) in the presence of lectins (0.625-10 μM) and after 24 h the cell viability was measured by MTT assay. Furthermore, the Viability/Cytotoxicity (Live/Dead kit), Cell proliferation (CFSE), Superoxide production (Mitosox kit), Mitochondrial membrane potential (ΔΨm) (Rhodamine 123), Lysosomal (acridine orange) and DNA damage (TUNEL assay) were investigated by flow cytometry.

DISCUSSION AND RESULTS: Both lectins significantly attenuated the H₂O₂-induced cytotoxicity in a dose-dependent way, the maximum protective effects were 96.85% (rCramoll) and 59.48% (pCramoll). The Live/Dead analysis showed a reduction in dead cells from 65.04% to 37.77% (pCramoll) and 13.90% (rCramoll). We next examined the signaling involved in lectins-mediated anti-death effects. The deleterious effects of H₂O₂ on cell proliferation were reduced 10.83% (pCramoll) and 24.17% (rCramoll). The lectins attenuated the excessive superoxide production, the collapse of ΔΨm, lysosomal and DNA damage that occurred in Vero cells exposed to H₂O₂. CONCLUSION: The results suggested that pCramoll and rCramoll blocked H₂O₂-induced cell death through decreasing reactive oxygen species, restoring ΔΨm, preventing lysosomal damage and DNA fragmentation, and thus promoting cell survival and proliferation.

Keywords: oxidative stress, lectins, cell biology.  
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