

EXPRESSION OF GYROXIN-LIKE IN CHO-DHFR⁻ CELLS.

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Gyroxin is a glycosylated multi-functional serine protease present in *C. d. terrificus* venom and belongs to Snake Venom Thrombin-Like Enzyme (SVTLE) family. Some SVTLEs are currently been used clinically for the treatment of thrombotic diseases and has a potential tissue sealant activity. The aim of the present work was to express the gyroxin in mammalian cells (CHO-DHFR⁻) in order to obtain its correct folding and proper posttranslational modifications. To accomplish this goal the gyroxin sequence coding to mature toxin (238 amino acids and 6 S-S bounds), was cloned into a dicistronic expression vector (pED) carrying a signal peptide to promote the protein secretion into the medium. CHO-DHFR⁻ cells were transfected and the level of methotrexate (MTX) in the culture medium was raised stepwisely, promoting gene amplification and increasing expression levels of the recombinant protein. Analysis using RT-PCR of some clones with and without MTX revealed the presence of DHFR and gyroxin mRNA. Western Blotting analysis is being performed to detect the gyroxin protein.

KEY WORDS: gyroxin, expression, *Crotalus*, serine protease, mammalian cells.

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