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FUNCTIONAL STUDY OF CRAC AND CARC PEPTIDES DERIVED FROM E. COLI ALPHA HEMOLYSIN

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PALABRAS CLAVE: Hemolysin, Immunotoxin, Peptides.

Escherichia coli alpha hemolysin (HlyA) is a pore-forming protein which belongs to the family of 'Repeat in toxins'(RTX). The CRAC domain refers to the Cholesterol Recognition/interaction Aminoacid Consensus sequence. The CARC domain is similar to the CRAC sequence, but exhibits the opposite orientation along the polypeptide chain.
The aims of this work were to study the participation of CRAC and CARC in the stabilization of HlyA monomers in membranes by their interaction with cholesterol, to evaluate the role of Y347 in the interaction with membrane, and finally to find a cytotoxic peptide for the construction of an immunotoxin.

On the basis of experimental data and structural predictions, six peptides derived from HlyA were synthesized: PEP 1: transmembrane domain described as hemolytically active; PEP 2: also a transmembrane domain which sequence corresponds to a cholesterol binding domain (CARC); PEP3: similar to PEP2 but with residue Y347 substituted by A; PEP4: similar to PEP2 but with a CRAC sequence; PEP5 and PEP6 correspond to CARC sequences located near the acylation sites. Peptides were synthesized by the solid phase peptide synthesis method (Fmoc strategy), purified by HPLC (C-18 column), the molecular mass was determined by mass spectrometry and peptide structure by circular dichroism. The hemolytic activity of peptides was measured using human erythrocytes and inhibition of hemolytic activity assays were performed pre-incubating erythrocytes with peptides and then adding them to wild type toxin.

Results describe PEP2 as hemolytic, which is promising and encourage us to use it in the design of immunotoxins. PEP3 was found not to be hemolytic suggesting residue Y347 is fundamental for the interaction of HlyA with lipidic membranes. PEP4 was found not to be hemolytic, which implicates the CRAC sequence added was unfavorable for peptide activity. PEP 6 competes with HlyA for binding sites in erythrocytes.

EVIDENCIAS BIOQUÍMICAS Y MOLECULARES DEL EFECTO DE COMPUESTOS NATURALES SOBRE UN NOVEDOSO BLANCO TERAPÉUTICO PARA ATEROSCLEROSIS

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PALABRAS CLAVE: Aterosclerosis, Aceite de cáscara de mandarina, Metabolismo lipídico.

La aterosclerosis es una enfermedad cardiovascular caracterizada por un engrosamiento de las paredes arteriales debido al depósito de lípidos, principalmente el colesterol (Col), y a una respuesta inflamatoria crónica promovida por macrófagos y células espumosas. Los niveles de Col plasmático se regulan por mecanismos como la síntesis de novo del Col o vía del mevalonato (VM). En las primeras etapas de la VM (reacciones pre-escualeno) se generan isoprenoides no esteroideos como ubiquinona, dolicol y grupos prenilos. La enzima óxido escualeno ciclasa (OSC) es quien cataliza la ciclación del escualeno para formar lanosterol, que es el primer componente cíclico de la VM del cual derivan todos los esteroides. La inhibición de la OSC representa un blanco terapéutico muy prometedor como agente hipocolesterolemiante debido a que interviene en una reacción post-escualeno, lo que implicaría inhibir la síntesis de Col sin afectar la de moléculas no esteroideas. Asimismo, su inhibición parcial produce oxiesteroles, que son fuertes ligandos del receptor nuclear LXR involucrado en la regulación de la expresión de enzimas de la vía de síntesis de triglicéridos (GPAT) y de proteínas ABC responsables del transporte reverso del Col. El aceite de cáscara de mandarina (ACM) posee compuestos naturales, mayoritariamente limoneno (Li), con potencial actividad hipocolesterolemiante y antiaterogénica. Objetivo: Evaluar el efecto del ACM sobre el metabolismo lipídico en hepatocitos y macrófagos, células que cumplen un rol fundamental en la