hope that these results have a relevant impact on the understanding of how bioactive compounds act in microbial cells and in the pursuit for proposals of mechanisms at the molecular level.

C14 - PE/PG DEMIXING INDUCED BY A SYNTHETIC MASTOPARAN-LIKE PEPTIDE IN MODEL MEMBRANES

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L1A, a synthetic peptide, is a potent Gram-negative antibacterial without being hemolytic. We have shown that the N-terminus acetylation of L1A (ac-L1A) enhanced the lytic activity in anionic vesicle compared to L1A which was correlated to its capability to insert into and disturb lipid packing of model membranes. We have evaluated the impact of L1A and ac-L1A, on model membrane that mimic the cytoplasm membrane of gram-negative bacteria, *e.g. E. Coli,* that contain mainly phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) using a variety of techniques. Circular dichroism experiments showed that the reduction of N-terminus charge did not influence its affinity to 3POPE:1DOPG large vesicles; however, the percentage of alpha-helix was higher for ac-L1A than for L1A. Further we employed differential scanning calorimetry to explore the thermotropic changes induced by these peptides. Thermograms of pure mixed lipids MLV, undergoes a gel to liquid-crystalline transition at 15 °C with a shoulder at ~17 °C. In the presence of both peptides, the transition peak was shift to higher temperature indicating that the incorporation of peptides induced lipid perturbation. ac-L1A was, however, able to induce higher phase separation with two symmetric phase transitions. Ac-L1A more deeply inserted into monolayers at constant area compared with L1A, inducing surface pressure changes that surpassed the lateral pressure of vesicles. Visualization of morphological domains change of lipid monolayer by fluorescence microscopy showed that the presence of both peptides disordered the hydrophobic chains preventing the formation of stiff films. All the results agree in that both peptides disrupt the lipid packing and that ac-L1A was able to cluster anionic lipids more efficiently.

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C15 - THE EFFECT OF SIZE DISTRIBUTION ON THE CYTOTOXICITY OF LIPID NANOPARTICLES

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A nanobiotechnology has contributed to the promising path in the development of new clinical treatments. The dynamism of colloidal systems becomes useful and, especially, due to the lack of physical techniques, which can be correlated with their biological performance, emphasizing the size of nanoparticles are directly related to biodistribution and cellular interaction. Thus, the objective of this work was to develop lipid nanocarriers based on nanostructured lipid carriers (NLC) and nanoemulsion (NE) formulations composed of copaiba oil (*Copaifera duckei*), which presents anti-inflammatory, antibacterial, antifungal and analgesic properties[1]. These systems present different average particle sizes, and this parameter was used to investigate its effect on the *in vitro* cytotoxicity. The structural characterization was carried out by Dynamic light scattering (DLS) in terms of size (nm), polydispersity index (PDI) and Zeta potential (mV); Nanoparticle tracking analysis (NTA) was employed to obtain the nanoparticle concentration (part/mL) as well as the particle size distribution of the formulations; and pH of the lipid nanoparticles was also quantified. NLC formulations showed particle size around 207.8 nm (DLS) and 141.4 nm (NTA), PDI close to 0.19 and Zeta values of -27.1 mV. NE formulations presented size around 128.7 nm (DLS) and 99.4 nm (NTA), PDI close to 0.18 and Zeta values of -20.5 mV. The amount of lipid nanoparticles was $5.17x10^{13}$ for NLC and $1.75x10^{14}$ for NE. Finally, the pH of NE was found more acid (3.35) than of NLC (5.65). Considering these clear differences in the structural properties of such copaiba-based nanostructured systems, cell viability assays will be performed for the analysis of cytotoxicity in fibroblast and mammary adenocarcinoma cells, in order to elucidate the size distribution effect on the further therapeutic action of these formulations.

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C16 - THE INFLUENCE OF pH AND ZETA POTENTIAL IN THE ANNEXIN V INCORPORATION INTO LIPOSOMES

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The mineralization process is mediated by osteoblasts that control the deposition of the extracellular matrix and the release of matrix vesicles (MVs), which serve as the initial sites for hydroxyapatite formation. Annexin V (AnxA5) is an acidic phospholipid-dependent Ca^{2+}-binding protein, which acts as Ca^{2+}-channel in the MVs' membrane. Herein, we describe the preparation of proteoliposomes composed of dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylserine (DPPS) harboring AnxA5. The aim is understanding the influence of pH and zeta potential (from 3 to 9) in the incorporation of AnxA5 into DPPC:DPPS 9:1 (molar ratio) liposomes (100 nm, 1.5 mg/mL). For pH range from 3 to 6, liposomes and proteoliposomes were produced in 100 mmol/L Acetate buffer. For pH from 7 to 9 vesicles were formed in 100 mmol/L Tris-HCl buffer. AnxA5 has incubated with liposomes (1:100 protein:lipid ratio) during 20h, at 25 °C under gentle stirring. Then, the mixture was ultracentrifuged at 100,000xg during 1 h, at 4 °C. The pellet containing proteoliposomes was resuspended in the initial volume of each buffer. The best yields of protein incorporation were obtained at pH 5 and 4 (113.8 and 16.6 μg/mL, respectively). In the other pHs, no more than 10 μg/mL of protein was incorporated. Zeta potential analysis were acquired to investigate the isoelectric point (IP) of the purified protein, DPPC:DPPS-liposome and DPPC:DPPS-proteoliposome harboring AnxA5. The analysis was performed in the same range of pH. AnxA5 has showed the IP at pH 3.1 and DPPC:DPPS-liposome and proteoliposomes exhibited IP of 3.5 and 4.1, respectively. It can be observed that when AnxA5 is present in the vesicles there is a Zeta Potential displacement to more negative values, from -30 mV for liposomes to – 40mV for proteoliposomes. This set of data indicate that AnxA5 binds at positives charge of liposomes, resulting in proteoliposomes with more negative Zeta Potential.

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C17 - Effects of Pore-forming toxins (PFTs) on oxidized model membranes represented by Giant Unilamellar Vesicles (GUVs)

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Sticholysin I (St1) and II (St2) are polypeptides isolated from the Caribbean sea anemone Stichodactyla Helianthus, with a molecular mass of 19401 and 19290 Da, with hemolytic and pore-forming properties in membranes (LANIO et al., 2000). It has been previously determined that the activity of membrane pore formation is related to the N-terminal insertion in the bilayer and lipid phase coexistence must play a role too (ROS et al., 2013). As PFTs, both toxins are considered to have potential appliance in parasitic and tumor diseases (MARTÍNEZ et al., 2007). In this work, we have investigated GUVs composed of non-oxidized POPC and POPC hydroperoxide (POPC-OOH) interacting with ST1 and ST2 by optical microscopy. The results did not reveal a significant PFT-membrane interaction such that no membrane destabilization was observed over incubation time of 20 min. On the other hand, when GUVs were made of mixtures of POPC or POPC-OOH and Sphingomyellin (SM), optical contrast fading was noticed indicating an increase in lipid bilayer permeability due to pore formation. Of note, neither micron-sized pores were observed nor membrane disruption. Further, the results also pointed out vesicles composed of oxidized lipids and SM have a much faster pore forming capacity. Therefore, our results thus suggest that membranes containing -OOH and SM promote the insertion of toxins due to their great fluidity, facilitating the insertion of the TFPs and their differentiation, leading to the formation of pores.

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