CHOLESTEROL IS CRITICAL FOR FLAVIVIRUS FUSION MECHANISMS

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Yellow fever and dengue are icosahedral enveloped RNA viruses that enter cells by endocytosis. Membrane fusion, mediated by glycoprotein E, is triggered by low pH inside the endosome. In this work, we evaluated through spectrofluorometric analysis, the requirement for specific lipids to viral fusion using a liposomal model system (ANTS/DPX) that allows observation of aqueous content mixing. Dengue virus fused efficiently with receptor-free liposomes consisting of phospholipids and cholesterol, suggesting that receptor interaction is not required for fusion. Under optimal conditions, 30 to 40% of fusion was mediated by DEN2 indicating a high mixing level. We used methyl-beta-cyclodextrin, a drug that efficiently depletes cholesterol from membranes, to investigate the role of envelope cholesterol in the *Flavivirus.* Pretreatment of virions with 10 mM methyl-beta-cyclodextrin efficiently depleted envelope cholesterol and significantly reduced dengue and yellow fever infectivity, suggesting that membrane cholesterol is a key component for flavivirus entry. Our results show that fusion is strongly dependent on the presence of cholesterol in the target membrane, and sphingomyelin does not seem to play an essencial role in the process.

Supported by: CNPq, CAPES, FAPERJ, FUJB/UFRJ AND PRONEX.

Keywords: Flavivirus, Cholesterol, Membrane Fusion.