Using the Fluorescence Lifetime Imaging Microscopy (FLIM) to Investigate Rhinovirus B-14 (RV-B14) Entry Into the Cells

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INTRODUCTION: Cells control their pH creating different proton concentrations in different organelles. Many viruses are used that, being transported to intracellular sites in which low pH helps its disassembly and delivery of the genetic material. These processes are well understood for some viruses, such as influenza, but for others little is known, such as rhinoviruses. The Rhinovirus is the major cause of colds, exacerbating symptoms of serious illnesses such as asthma and COPD. Little is known, for example, about structural changes that occur in the viral capsid during entry into the cell, and the importance of pH variations in this process has been studied only in vitro. OBJECTIVE: We studied the importance of the pH variations during the entry of the Rhinovirus B-14 cells, investigating in real time the structural changes in the capsid. MATERIALS AND METHODS: In this study, we label the viral capsid with FITC, probe sensitive to pH, and use Fluorescence Lifetime Imaging Microscopy (FLIM) and analysis by phasors to study the lifetime of that probe in each pixel of the images, showing how fluctuate pH and capsid conformation. DISCUSSION AND RESULTS: As the virus experience more acidic solutions, the lifetimes tend to zero, with straight course, but before completing this path, return to higher values, with arched trajectory. Monitoring the virus entry into the cell, we identified lifetimes similar to FITC at pH 8, intermediates and some populations with linear distribution tending to zero, but with no arc trajectory. CONCLUSION: These data indicate that, at decreasing pH, the viral capsid undergoes structural changes similar to those suggested for the stabilization of the particle after binding to its receptor. These changes are translated by increasing the quenching process, which occurs by the approach of FITC molecules, and demonstrate the influence of pH decreasing during virus disassembly.

Key words: Rhinovirus, Fluorescence Lifetime Microscopy, virus-cell interactions.