Cell Death Photoinduced Damage in Lysosomes Triggered by Acridine Orange

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INTRODUCTION: In order to develop new photosensitizers (PS) for Photodynamic Therapy (PDT) it is important to localize the damages induced by it and to understand the subsequent mechanisms of cell death **OBJECTIVES:** This study aims to analyze Acridine Orange (AO) as a PS for PDT, because it is well known to target lysosomes and to be photoactive. **MATERIAL AND METHODS:** In order to understand the sub-cellular localization profile of AO as a function of time, we performed fluorescence microscopy analysis regarding to state-state and time resolution modes. In addition, we performed the MTT reduction assay to determine the cytotoxic AO effects induced by light dose of 2J/cm^2(466nm) for all times of incubation (10, 60 and 180 minutes), using human keratinocytes (HaCaT) as cell model. **RESULTS AND DISCUSSION:** Within 10 minutes of incubation, AO homogeneously accumulated in the cytoplasm with minor parallel accumulation on the cell membrane. After 1-hour, we observed conspicuous AO accumulation represented by well-defined cytoplasmic punctuations, which partially changed to a more homogenous intracellular distribution after 3-hours. By using a time-resolved confocal microscope, we showed similar distribution profile as observed by epifluorescence, but with an additional information that the AO´s fluorescence lifetime was the same regardless of its intracellular distribution. According to the cell viability assay, the AO cytotoxic effects photo-induced significantly increased in a dose-dependent manner. Interestingly, despite of different incubation-times (10 or 60 minutes) lead to distinct AO intracellular distribution, the phototoxicity ascribed to AO did not significantly differ with similar DL_{50} (0.4 and 0.35 µM). **CONCLUSION:** So far, our findings corroborate to recent literature prompting us to investigate further the AO cytotoxic effects at molecular level by identifying typical signs of cell death and performing analysis of these pathways inhibited by RNAi.

Key words: Photodynamic Therapy, Acridine Orange, Cell Death