**Title: Mechanistic Study of the Photodynamic Inactivation of *Candida albicans* by phenothiazinium photosensitizers**

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**Abstract:**

Photodynamic antimicrobial chemotherapy (PACT) is a promising method which combines a nontoxic photosensitizer (PS) with visible light to cause selective killing of microbial cells. We investigated the susceptibilities of *Candida albicans* to PACT with two phenothiazinium derivatives, new methylene blue N (NMBN) and the novel pentacyclic phenothiazinium S137 in combination with red light. The effectiveness of each PACT was determined based on cell survival. Additionally, the mechanisms of action were investigated by flow cytometry with several fluorescent probes. Light exposures alone (3 to 14 J $cm^{-2}$) and treatment with the PSs (2.5 µM) in the absence of light did not kill *C. albicans*. PACT both with NMBN and S137 killed the cells in a fluence-dependent manner. PACT with NMBN and S137 resulted in a reduction in the survival of the cells from 0.83 log (3 J $cm^{-2}$) to 5.18 logs (14 J $cm^{-2}$) and from 3.70 logs (3 J $cm^{-2}$) to 4.83 logs (14 J $cm^{-2}$), respectively. Propidium iodide (PI), a marker of cell death (it only penetrates cells with severe membrane lesions). PACT with NMBN resulted in PI staining of the cells in a fluence-dependent manner from 30% to 90 %. PACT with S137 resulted in PI staining from 90% to 99%. In metabolically inactive cells, FUN-1 remains in the cytoplasma, displaying a green fluorescence, while in active cells it is processed which results in the formation a red fluorescence, accompanied by reduction in the green cytoplasmic fluorescence. The PACT with FS NMBN and S137 increased green fluorescence and this increase was directly proportional to the survival of *Candida* cells. The ROS were assessed using dihydroethidium and dihydrorhodamine 123 which are relatively specific for superoxide and hydrogen peroxide, respectively. These probes are oxidized in the presence of ROS emitting fluorescence. PACT with both PS increased the fluorescence (with both probes) in a fluence-dependent manner. The mitochondrial membrane potential was assessed using the fluorescent probe JC-1 which is a cationic dye that exhibits potential-dependent accumulation in mitochondria; mitochondrial polarization is thus indicated as an increase in the red/green fluorescent intensity ratio. PACT with both PS decreased the ratio between the fluorescent red/green indicating a loss of mitochondrial membrane potential. The results obtained showed that the markers can be used to identify the processes and cellular structures that are affected by PACT in *C. albicans*.

**Key-words:** *Candida albicans*, photodynamic antimicrobial therapy, phenotiazinium photosensitizers, fluorescent probes

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