Methylene Blue Photodynamic Therapy Induces Selective and Massive Cell Death in Human Breast Cancer Cells

dos Santos, A.F.; Terra, L.F.; Wailemann, R.A.M.; Oliveira, T.C.; Gomes, V.M.; Mineiro, M.F.; Meotti, F.C.; Bruni-Cardoso, A. Baptista, M.S.; Labriola, L.

Biochemistry Department, Chemistry Institute, University of São Paulo (USP), São Paulo, Brazil

Breast cancer is a worldwide health problem for women, presenting high recurrence due to primary treatment failure. Photodynamic therapy (PDT), which causes tissue destruction by visible light in the presence of a photosensitizer and oxygen, is a promising alternative to cure cancer. The efficacy of PDT to treat breast tumors as well as the mechanisms that lead to cell death remain unclear. In this study, we assessed the cytotoxic potential of PDT using methylene blue (MB-PDT) in three breast epithelial cell lines that represent non-malignant conditions and different molecular subtypes of breast tumors. Cells were incubated in the absence or presence of MB and irradiated or not at 640 nm with 4,5J/cm^2. We used a combination of imaging and biochemistry approaches to assess the involvement of classical autophagic, apoptotic and necroptotic pathways in mediating the cell-deletion action of MB-PDT. The role of these pathways was investigated using specific inhibitors, activators and gene silencing. MB-PDT induces massive cell death of tumor cells. However, non-malignant cells were significantly more resistant to therapy. While nuclear staining showed no signs of apoptosis, in two of the cell lines caspase-activities were involved in cell death since their inhibition modulated the MB-PDT effect. A significant increase in LC3-II and acidic vesicle formation was observed upon MB-PDT. Depending on the cell line, autophagy resulted in either cytoprotection or cytotoxicity. Inhibiting RIPK1 or caspase-8 led to cell protection and increased cell susceptibility to MB-PDT respectively, showing that necroptosis is an important pathway activated upon MB-PDT. Additionally, when using a physiological 3D culture model that recapitulates relevant features of breast tissue morphology, we found that MB-PDT differential action in killing tumor cell was even higher. Finally, our observations underscore the potential of MB-PDT as a highly efficient strategy to safely treat breast cancer and possibly other types of tumors.

Keywords: MB-PDT, breast cancer, cell death
Support: FAPESP, CNPq, CAPES