Manganoporphyrin MnTMPyP enhances ascorbate-induced cytotoxicity in pancreatic cancer cells via generation of H$_2$O$_2$

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High dose, pharmacological (i.v.) ascorbate is being investigated as a non-conventional therapy in pancreatic and other cancers. Ascorbate-induced cytotoxicity is thought to be mediated by generation of H$_2$O$_2$ in the extracellular fluid with ascorbate and its radical acting as electron donors. Our goal is to increase the effectiveness of this therapy by combining it with redox active molecules to increase the flux of H$_2$O$_2$. We hypothesize that the SOD mimetic Mn (III) tetrakis (1-methyl-4-pyridyl) porphyrin (MnTMPyP) will increase the rate of oxidation of ascorbate thus increasing the flux of H$_2$O$_2$. Addition of MnTMPyP to a near-neutral solution of ascorbate increased the level of ascorbate radical, consistent with an increased rate of ascorbate oxidation and formation of H$_2$O$_2$. In vitro, exposure of human pancreatic cancer cell lines MIAPaCa-2 and AsPC-1 to 1 µM MnTMPyP or 1 mM ascorbate showed minimal effects on clonogenic survival. However, when combined, clonogenic survival was decreased synergistically. Inclusion of 100 U/mL PEG-SOD or 100 MOI AdEcSOD did not alter clonogenic survival indicating that superoxide is not directly involved in toxicity; addition of 120 U/mL PEG-catalase reversed cytotoxicity, consistent with a major role for H$_2$O$_2$. MnTMPyP and ascorbate decreased procaspase-3 while increasing Annexin V/propidium iodide staining, consistent with apoptosis. LC3-II increased with upon treatment indicating autophagy. We conclude that: 1) MnTMPyP increases the rate of oxidation of ascorbate; leading to 2) an increase in the level of ascorbate radical; 3) an increase in the flux of H$_2$O$_2$; and 4) an increase in the toxicity of ascorbate in tumor cells. Taken together, these preclinical findings suggest that MnTMPyP may be a promising adjuvant to pharmacological ascorbate to treat pancreatic and other cancers.