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ASCORBATE INDUCES AUTOPHAGY IN PANCREATIC CANCER

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Ascorbate (ascorbic acid, vitamin C) is one of the early, unorthodox treatments for cancer. The evidence upon which people base the use of ascorbate in cancer treatment falls into two categories: clinical data on dose concentration relationships, and laboratory data describing potential cell toxicity with high concentrations of ascorbate *in vitro*. Clinical data show that when ascorbate is given orally, fasting plasma concentrations are tightly controlled by decreased absorption, increased urine excretion, and reduced ascorbate bioavailability. In contrast, when ascorbate is administered intravenously, concentrations in the millimolar level are achieved. Thus, it is clear that intravenous administration of ascorbate can yield very high plasma levels, while oral treatment does not.

Pharmacological ascorbate selectively kills some cancer cell types including pancreatic cancer. Cell death is dependent on H₂O₂ formation. H₂O₂ generation is dependent on ascorbate concentration and incubation time, and displays a linear relationship with ascorbate radical formation, ascorbate being the electron-donor. Thus, pharmacological ascorbate concentrations produce extracellular H₂O₂, which diffuses into cells causing cell death via oxidative stress. The role of autophagy in cellular responses to oxidative stress is as yet unclear. Reactive oxygen species (ROS), like H₂O₂, can induce autophagy, which may contribute to cell death. In contrast, autophagy may play a protective role of in ROS-mediated necrosis.

Our recent study suggests that ascorbate may lead to death through a unique caspase-independent autophagy pathway. Autophagy is characterized by accumulation of autophagosomes that fuse with lysosomes to form autolysosomes. Activation of this pathway can be detected by the processing of LC3 to the lipidated form referred to LC3-II. Pancreatic cancer cells treated with ascorbate demonstrate an increase in LC3-II immunoreactive protein. This increase in LC3-II is reversed by pretreatment of the cells with catalase suggesting that the ascorbate-induced induction of autophagy is mediated by H₂O₂. To confirm this finding, we generated human pancreatic cancer cells expressing a fusion product of LC3 and GFP to detect the induction of autophagy. Both ascorbate and H₂O₂ significantly increase GFP fluorescence. The increased ascorbate-induced GFP fluorescence in these cells is also blocked by catalase.

Thus, oxidative stress mediated by hydrogen peroxide, is a mechanism of ascorbate-induced cytotoxicity. In addition, an autophagic mechanism may also be involved, as an increase in

LC3 protein is observed in cells treated with ascorbate. As mentioned, the role of autophagy in cellular responses to oxidative stress is as yet unclear. If induction of autophagy contributes to cell death in ascorbate-induced cytotoxicity then both a peroxide-mediated mechanism and autophagic-mediated mechanism could potentially be enhanced to induce more cancer cell killing with peroxide.

Therapeutic interventions designed to inhibit hydroperoxide detoxification combined with manipulations that increase hydroperoxide production could be used with ascorbate to potentially increase autophagy and preferentially kill tumor cells versus normal cells *via* metabolic oxidative stress. This could be achieved by inhibiting the antioxidant enzymes that prevent or repair the damage caused by peroxide. Catalase and peroxidases convert H_2O_2 into water. Catalase does not need co-factors to function, but glutathione peroxidase (GPx) requires several cofactors and secondary enzymes. As seen in the antioxidant enzyme scheme (Fig. 1), there is a clear rationale for ascorbate combined with certain cytotoxic agents.

If H_2O_2 removal is inhibited, then pancreatic cancer cells should be killed because of direct toxicity resulting from H_2O_2 -mediated damage. There are a number of compounds that can inhibit the removal of H_2O_2 . For example, 1,3 bis (2-chloroethyl)-1-nitrosurea (BCNU) is a clinically used anticancer drug that inhibits glutathione reductase (GR) leading to inhibition of H_2O_2 removal *via* the glutathione peroxidase system. Buthionine sulfoximime (BSO) is a competitive inhibitor of a glutathione synthetic enzyme γ -glutamyl cysteine synthetase (γ -GCS), leading to glutathione depletion and resulting in the inability of GPx to remove H_2O_2 . 2-deoxy-D-glucose (2DG) is a relatively non-toxic analog of glucose that competes with glucose for uptake *via* the glucose transporters as well as being phosphorylated by hexokinase at the entry point to glycolysis. Competition between 2DG and glucose is thought to cause inhibition of glucose metabolism, thereby creating a chemically induced state of glucose deprivation resulting in inhibition of hydroperoxide detoxification. Combinations of these chemical inhibitors of glucose and hydroperoxide metabolism would be predicted to enhance ascorbate toxicity.

If induction of autophagy contributes to cell death in ascorbate-induced cytotoxicity, then enhancement of the autophagic-mediated mechanism could also be utilized to potentially induce more cancer cell killing. For example, would the class III phosphatidylinositol 3-phosphate kinase autophagy inhibitor 3-MA, enhance or inhibit ascorbate-induced cytotoxicity? Also, would the inhibition of the mammalian target of rapamycin (mTOR) with rapamycin synergize with ascorbate? Further studies are needed to determine if the autophagic response of pancreatic cancer cells to ascorbate treatment is an indication of cell death or a protective response to the treatment allowing the recycling of proteins and cellular components.

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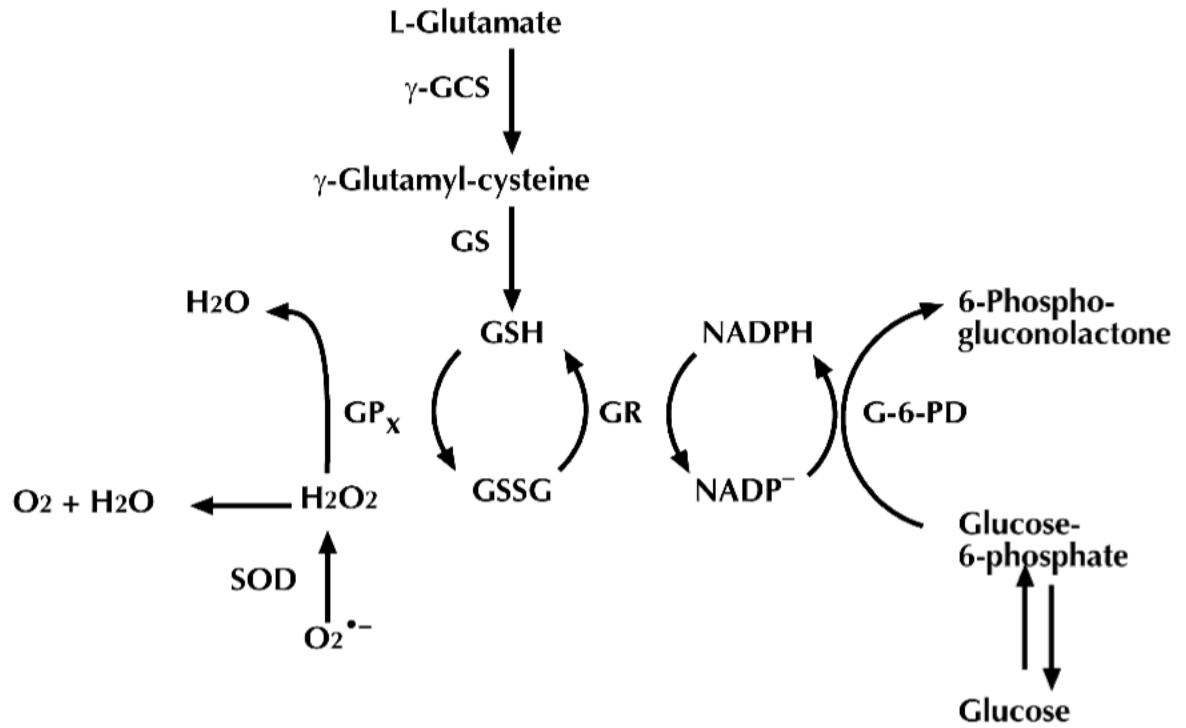


Figure 1. Antioxidant enzyme schematic

GSH = glutathione; GSSG = glutathione disulfide; GR = glutathione disulfide reductase; G-6-PD = glucose-6-phosphate dehydrogenase; γ GCS = γ -glutamylcysteine synthetase; GS = glutathione synthetase.