TARGETS AND MECHANISMS OF ACTION ASSOCIATED WITH LASER

MEDIATED PHOTOSENSITIZATION

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I. INTRODUCTION

Photodynamic therapy (PDT) is the treatment of malignant lesions with visible light following the systemic administration of a tumorlocalizing photosensitizer. Hematoporphyrin derivative (HpD) and a purified component called Photofrin II are currently used in clinical PDT and this therapy continues to show promise in the treatment of solid tumors. However, it is clear that PDT is still at an early stage in its development. In this chapter, we will examine molecular, cellular, and in-vivo mechanisms related to PDT.

II. SUBCELLULAR TARGETS AND MOLECULAR RESPONSE ASSOCIATED WITH PDT

Numerous studies have demonstrated that PDT is extremely effective in generating cytotoxic damage to subcellular organelles and biomolecules. Photodegradation of lipids, proteins and nucleic acids can routinely be observed following porphyrin photosensitization. Membrane damage can lead to inhibition of transport of amino acids and nucleosides, increased permeability and rupture of lysosomes, as well as marked contraction and rupture of mitochondria. Enzymes bound to hydrophobic regions of mitochondria (membrane bound) are extremely sensitive to PDT. At the level of nucleus PDT can produce single strand breaks in DNA, sister chromatid exchange and chromosome aberrations. Even though a large spectrum of specific types of subcellular damage have been documented following PDT, the actual target site(s) for cytotoxicity has not been identified.

II.1. Differential Cell Photosensitivity Following Porphyrin Photodynamic Therapy

Interestingly, the mechanism of action for PDT in experimental tumors is thought to involve both direct tumor cell kill and direct tumor vessel damage. Vascular damage is also implicated in PDT-induced damage to normal tissues such as brain, intestine and skin. Several studies have indicated that malignant and normal cells accumulate similar levels of porphyrin during in-vitro incubation. Differences in cell photosensitiv-
direct comparison of level of photosensitization in cells with varying levels of DNA repair properties, as well as in cells which make up various components of the vasculature\textsuperscript{15}. Interestingly, while the human DNA repair deficient fibroblasts ataxia telangiectasia (AT) and xeroderma pigmentosum (XP) expressed extreme hypersensitivity to ionizing radiation and to ultraviolet radiation respectively compared to normal fibroblasts, survival curves for PDT resulted in similar levels of photosensitivity. These findings support the premise that non-nuclear damage, such as that induced in mitochondria and/or the plasma membrane, is of primary importance in terms of PDT-induced cytotoxicity\textsuperscript{6}. Porphyrin-induced photosensitization does not induce mutagenic\textsuperscript{5} or transformation\textsuperscript{16} activity in mammalian cells and these observations in cellular photosensitivity have been observed when components of the vasculature were examined\textsuperscript{15}. Specifically, bovine cells of endothelial, smooth muscle and fibroblasts origin were compared for porphyrin retention and photosensitivity. Bovine endothelial cells were considerably more sensitive than smooth muscle or fibroblast cells treated under identical conditions when assayed for viability using clonogenicity. The increased photosensitivity observed in endothelial cells can not be accounted for on the basis of cellular porphyrin content at the time of the treatment since all bovine cells accumulated similar levels of porphyrin. The results indicate that endothelial cell photosensitivity may play a role in the vasculature damage observed following porphyrin photodynamic therapy.

II.2. Stress Protein Production Following Photodynamic Therapy

Eucaryotic cells respond to a transient stress, such as heat shock, by inducing the synthesis of a specific set of highly conserved proteins known as the heat shock proteins (HSP)\textsuperscript{17}. These proteins are generally thought to play a role in protecting cells from subsequent stresses and/or in enhancing the recovery of injured cells. Some of these proteins are produced constitutively, while others are only synthesized under the influence of a variety of cellular stresses. It is become increasingly clear that expression of heat shock genes is not limited to cells that are undergoing acute stress\textsuperscript{18}. The eucaryotic genome also encodes proteins which are closely related in sequence to HSP-70, but which appear to be regulated distinctly. The synthesis of this second stress-responsive group of proteins, the glucose regulated proteins (GRP), is induced under a variety of conditions including glucose deprivation, anoxia, treatment of cells with glycosylation inhibitors or the calcium ionophore A23187\textsuperscript{19}.

Singlet oxygen generated via a Type II photochemical mechanism is thought to initiate most damage following Photofrin II mediated PDT\textsuperscript{20,21}. The treatment can induce stress proteins of both the heat shock (HSP) and glucose regulated (GRP) families. A comparison of the kinetics and characterization of photosensitizer-induced stress proteins with proteins induced by other oxidative stress, may suggest several complementary modes of action for PDT and provides a novel method for identifying oxidative species involved in PDT. An extensive time-dependent increase in GRP-78 gene expression was observed in RIF-1 cells first incubated with Photofrin II for 16 hours and then exposed to a dose of visible light which resulted in 20-30% survival level\textsuperscript{22}. The significance of this finding is currently unclear, although it is known that GRP-78 expression is strongly induced by agents which affect posttranslational processing events in the endoplasmic reticulum\textsuperscript{19}. The product of GRP 78 gene is a nonglycosylated protein with an apparent molecular weight of 78,000 daltons and is found in the lumen of the ER of most cell types. GRP-78 has been detected in association with proteins when their folding or assembly is incomplete or improper\textsuperscript{23}. The GRP-protein complex may prevent the secretion of abnormal proteins presumably maintaining them in soluble form until the stress condition is re-