World Academy of Science, Engineering and Technology
International Journal of Mathematical and Computational Sciences
Vol:14, No:12, 2020

Photobleaching Kinetics and Epithelial Distribution of Hexylaminoleuilinate Induced PpIX in Rat Bladder Cancer

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Abstract : Photodynamic therapy (PDT) is a treatment modality based on the cytotoxic effect occurring on the target tissues by interaction of a photosensitizer with light in the presence of oxygen. One of the major advances in PDT can be attributed to the use of topical aminolevulinic (ALA) to induce Protoporphyrin IX (PpIX) for the treatment of early stage cancers as well as diagnosis. ALA is a precursor of the heme synthesis pathway. Locally delivered to the target tissue ALA overcomes the negative feedback exerted by heme and promotes the transient formation of PpIX in situ to reach critical effective levels in cells and tissue. Whereas early steps of the heme pathway occur in the cytosol, PpIX synthesis is shown to be held in the mitochondrial membranes and PpIX fluorescence is expected to accumulate in close vicinity of the initial building site and to progressively diffuse to the neighboring cytoplasmic compartment or other lipophylic organelles. PpIX is known to be highly reactive and will be degraded when irradiated with light. PpIX photobleaching is believed to be governed by a singlet oxygen mediated mechanism in the presence of oxidized amino acids and proteins. PpIX photobleaching and subsequent spectral phototransformation were described widely in tumor cells incubated in vitro with ALA solution, or ex vivo in human and porcine mucosa superfused with hexylaminolevulinate (hALA). PpIX photobleaching was also studied in vivo, using animal models such as normal or tumor mice skin and orthotopic rat bladder model. Hexyl aminolevulinate a more potent lipophilic derivative of ALA was proposed as an adjunct to standard cystoscopy in the fluorescence diagnosis of bladder cancer and other malignancies. We have previously reported the effectiveness of hALA mediated PDT of rat bladder cancer. Although normal and tumor bladder epithelium exhibit similar fluorescence intensities after intravesical instillation of two hALA concentrations (8 and 16 mM), the therapeutic response at 8mM and 20J/cm2 was completely different from the one observed at 16mM irradiated with the same light dose. Where the tumor is destroyed, leaving the underlying submucosa and muscle intact after an 8 mM instillation, 16mM sensitization and subsequent illumination results in the complete destruction of the underlying bladder wall but leaves the tumor undamaged. The object of the current study is to try to unravel the underlying mechanism for this apparent contradiction. PpIX extraction showed identical amounts of photosensitizer in tumor bearing bladders at both concentrations. Photobleaching experiments revealed mono-exponential decay curves in both situations but with a two times faster decay constant in case of 16mM bladders. Fluorescence microscopy shows an identical fluorescence pattern for normal bladders at both concentrations and tumor bladders at 8mM with bright spots. Tumor bladders at 16 mM exhibit a more diffuse cytoplasmic fluorescence distribution. The different response to PDT with regard to the initial pro-drug concentration can thus be attributed to the different cellular localization.

Keywords : bladder cancer, hexyl-aminolevulinate, photobleaching, confocal fluorescence microscopy
Conference Title : ICSRD 2020 : International Conference on Scientific Research and Development
Conference Location : Chicago, United States
Conference Dates : December 12-13, 2020