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Research Paper

Dual roles of nitric oxide in the regulation of tumor cell response and resistance to photodynamic therapy

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ABSTRACT

Photodynamic therapy (PDT) against cancer has gained attention due to the successful outcome in some cancers, particularly those on the skin. However, there have been limitations to PDT applications in deep cancers and, occasionally, PDT treatment resulted in tumor recurrence. A better understanding of the underlying molecular mechanisms of PDT-induced cytotoxicity and cytoprotection should facilitate the development of better approaches to inhibit the cytoprotective effects and also augment PDT-mediated cytotoxicity. PDT treatment results in the induction of iNOS/NO in both the tumor and the microenvironment. The role of NO in cytotoxicity and cytoprotection was examined. The findings revealed that NO mediates its effects by interfering with a dysregulated pro-survival/anti-apoptotic NF-κB/Snail/YY1/RKIP loop which is often expressed in cancer cells. The cytoprotective effect of PDT-induced NO was the result of low levels of NO that activates the pro-survival/anti-apoptotic NF-κB, Snail, and YY1 and inhibits the anti-survival/pro-apoptotic and metastasis suppressor RKIP. In contrast, PDT-induced high levels of NO result in the inhibition of NF-kB, Snail, and YY1 and the induction of RKIP, all of which result in significant anti-tumor cytotoxicity. The direct role of PDT-induced NO effects was corroborated by the use of the NO inhibitor, L-NAME, which reversed the PDT-mediated cytotoxic and cytoprotective effects. In addition, the combination of the NO donor, DETANONOate, and PDT potentiated the PDT-mediated cytotoxic effects. These findings revealed a new mechanism of PDT-induced NO effects and suggested the potential therapeutic application of the combination of NO donors/iNOS inducers and PDT in the treatment of various cancers. In addition, the study suggested that the combination of PDT with subtoxic cytotoxic drugs will result in significant synergy since NO has been shown to be a significant chemo-immunosensitizing agent to apoptosis.

1. Introduction

Photodynamic therapy (PDT) is a therapeutic modality for certain diseases including cancer. PDT consists primarily of a photosensitizer (PS) and followed by light irradiation of a predetermined wavelength [1]. However, oxygen is an essential mediator of PDT [1,2]. The PDT-generated reactive oxygen species (ROS) and singlet oxygen ($^1O_2$) cause damage to the tumor tissues and cells by inducing necrosis and apoptosis. Optimally, the selective effect of PDT is through the localization of the photosensitizer in the desired region and the precise delivery of the light source to the treated areas. The PDT activity has its own limitations, for example, its effect on metastatic cancer lesions.

1.1. The photosensitizer (PS)

Most of the photosensitizers (PSs) used in cancer therapy belong to the protoporphyrin family and are based on a tetrapyrrole structure. An ideal sensitizer must have an absorption peak

Abbreviations: ABC, ATP-binding cassette; ABCG2, ATP-binding cassette sub-family G member 2; AIF, apoptosis inducing factor; ALA, aminolevulinic acid; BCC, basal cell carcinoma; BCG, Bacillus Calmette-Guerin; CG, cholangiocarcinoma; CTL, cytotoxic T-lymphocyte; DR4/DR5, TRAIL death receptors; EGF, epithelial growth factor; EMT, epithelial mesenchymal transition; FASL, fas ligand; FDA, food and drug administration; 5-FU, 5-fluorouracil; GI, gastrointestinal; GSNO, S-nitrosoglutathione; HBD, hematoporphyrine-derivative; iNOS, inducible nitric oxide synthase; L-NAME, L-N^G-Nitroarginine methyl ester; MAL, methylaminolevulinate; MDR, multidrug resistance; mPEG, monomethoxy-polyethylene glycol; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK, natural killer; $^3O_2$, molecular singlet oxygen; $^1O_2$, singlet oxygen; PARP, poly ADP ribose polymerase; Pba, pheophorbide a; PDT, photodynamic therapy; PS, photosensitizer; RIPT-1, receptor activity protein I; RKIP, Raf kinase inhibitor protein; ROS, reactive oxygen species; Ru (NO)(NO)(ONO)(pc), nitrosyl-phtalocyanin ruthenium complex; SCC, squamous cell carcinoma; SNAP, S-nitroso-N-acetylpenicillamine; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha; TRAIL, TNF-related apoptosis-inducing ligand; TNF-R1/R2, tumor necrosis factor receptor 1/receptor 2; UV, ultraviolet; YY1, Yin Yang 1

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between 600 and 800 nm (red to deep red). High wavelengths greater than 800 nm produce a limited source of photons since they are poor in exciting oxygen to its singlet state and, thus, reduce reactive oxygen species that are required for cytotoxic effects. The mechanism of tumor localization of PS has been investigated revealing the role of the leaky blood vasculature in cancers and the absence of drainage by the lymphatic system leading to retention [3]. Also, some PSs bind to low density lipoproteins and bind cancers overexpressing LDL receptors and, thus, are more directed on tumors [4]. Other reports also demonstrated the use of PSs covalently linked to binding agents directed at cancer bearing receptors on the tumor cell surface [5]. Such coupling agents include antibody molecules, antibody fragments, peptides, proteins, EGF, etc.

1.2. Light sources for radiation

Red and infrared radiation penetrate into tissues more deep and only in the range of 600 to 800 nm to generate singlet oxygen for toxicity [6]. The choice of light source is dependent on the PS used and is based on the PS absorption, the disease and its size. The fluence rate affects significantly the PDT response [7]. Both lasers and incandescent light sources have been used for PDT and result in similar effects [8]. More detailed analyses of light sources have been reviewed elsewhere [9–14].

1.3. Photochemistry

The light exposure on the PS undergoes a shift from the ground (singlet) state to an excited singlet state. The latter undergoes crossing to an excited triplet state and this can result in the formation of radicals (ROS) (Type I reactions) or transfer the energy to molecular singlet oxygen ($^3O_2$) to form singlet oxygen ($^1O_2$) (Type II reactions). Singlet oxygen is the predominant cytotoxic molecule in PDT [9].

2. Dual cytotoxic and cytoprotective roles of PDT

2.1. PDT-mediated cytotoxicity

Various PSs target different organelles and subcellular compartments and mediate cytotoxic effects, which will vary based on the targeting – and the sensitivity of the tumor cells to cytotoxic damage [13,15]. Three major types of cell death by PDT have been reported, namely, (1) apoptosis, (2) necrosis and (3) autophagy. Apoptosis is the major cell death mechanism induced by PDT [9,14].

2.2. PDT-mediated cytoprotection

Many cancer cells are not sensitive to PDT-mediated cytotoxicity. Tumor cells develop various mechanisms to protect them from cell death-induced by PDT and many other cytotoxic agents. For instance, certain cancer cells have high levels of antioxidants [16]. Others have overexpression of detoxifying enzymes for ROS [17] and may have protective genes induced by PDT and/or overexpress several anti-apoptotic gene products [18–20]. A more detailed analysis on the mechanisms discussed above would be reported below.

3. Clinical applications of PDT in a variety of human cancers

Historically, Dougherty et al. [21] reported the first clinical study of the application of PDT in patients with a variety of malignant diseases. They treated the patients with PDT with a hematoporphyrine-derivative (HBD). They achieved complete and partial responses in 111 out of 113 treated cancer patients. These initial successful findings of the application of PDT in cancer was followed by hundreds of clinical trials [9,22,23]. PDT was most effective on the surface of lesions due to the limited penetration of the light source deep into the tissues; the range of tumor destruction did not overall exceed one centimeter. Briefly, a few examples of the therapeutic applications of PDT in various cancers are presented.

Còrdoba et al. [24] and Nestor et al. [25] reviewed the response of PDT treatment in premalignant and malignant skin tumors. Noteworthy, PDT was approved in the USA, Canada and Europe for its use in actinic keratosis and also in the European Union and Canada for basal cell carcinoma (BCC). In actinic keratosis, randomized controlled trials reported complete response rates (82–100%) for PDT with aminolevulinic acid (ALA-PDT) or methylaminolevulinate (MAL-PDT) as compared to 67 to 100% for cryotherapy and 74–94% for the application of 5-FU cream at 12 and 24 months [26,27]. In BCC, PDT was superior to cryosurgery or surgery for a selected subset of patients. Also, PDT actinic is a superior cosmetic outcome compared to surgery [28,29]. The use of MAL-PDT was found to be a safe and effective treatment for BCC in patients with Gorlin's syndrome and its efficacy is correlated to the thickness of the region [30]. PDT was also found to have chemo-preventive activity in patients with the Gorlin's syndrome [31].

PDT has been employed in the treatment of head and neck cancer, successfully [32]. Of interest, the study evaluated PDT treatment of patients with advanced diseases and not responding to tumor treatments. They applied Foscan-mediated PDT in 128 patients with a single session of PDT. There was a remarkable response in tumor destruction and complete local tumor clearance [33]. These findings suggest that PDT may be an alternative treatment for patients with early head and neck tumors.

Tumors of the digestive system have been grouped into PDT of the esophagus [34] and tumors beyond the esophagus. The U.S. FDA approved photofrin-mediated PDT for patients with Barret's esophagus and high grade dysplasia who did not undergo surgery [34]. PDT has been applied to other GI digestival tumors under the stomach [35,36], cholangiocarcinoma (CG) [37], with a therapeutic response on unresectable pancreatic cancers [38], and on colon or rectal cancers [39,40].

Intraperitoneal (ovarian, gastrointestinal, sarcoma) have been treated with PDT [41]. There was a suggestion that the median survivals of two years for ovarian cancer and one year for gastrointestinal cancer have been beneficial by PDT compared to controls.

Several reports have shown that the results of PDT treatment of prostate cancer. These studies established the potential use of PDT in prostate cancer and toxicity was considered as a determining factor [42–44].

Superficial bladder cancer is a good target for PDT. Long-term desirable responses of 20–60 of patients who were treated and many of those patients had recurrent disease following BCG treatment [45,46]. While PDT treatment for bladder cancer has been approved in the EU and Canada, it is not yet approved by the U.S. FDA.

In non-small cell lung cancer, the results of PDT treatment are encouraging [47,48]. In patients with malignant pleural mesothelioma, a randomized phase III study compared PDT with surgery and the findings demonstrated the benefit of PDT over surgery [49].

Promising clinical findings of PDT in brain tumors were reported [50,51]. However, more phase III clinical trials are needed to place PDT as superior to other therapeutics in certain cancers.