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Site-directed photoproteolysis of 8-oxoguanine DNA glycosylase 1 (OGG1) by specific porphyrin-protein probe conjugates: A strategy to improve the effectiveness of photodynamic therapy for cancer

Kimberly A. Conlon *, Miguel Berrios

Department of Pharmacological Sciences, School of Medicine, University Hospital and Medical Center, State University of New York, Stony Brook, NY 11794-8651, USA

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Abstract

The specific light-induced, non-enzymatic photolysis of mOGG1 by porphyrin-conjugated or rose bengal-conjugated streptavidin and porphyrin-conjugated or rose bengal-conjugated first specific or secondary anti-IgG antibodies is reported. The porphyrin chlorin e6 and rose bengal were conjugated to either streptavidin, rabbit anti-mOGG1 primary specific antibody fractions or goat anti-rabbit IgG secondary antibody fractions. Under our experimental conditions, visible light of wavelengths greater than 600 nm induced the non-enzymatic degradation of mOGG1 when this DNA repair enzyme either directly formed a complex with chlorin e6-conjugated anti-mOGG1 primary specific antibodies or indirectly formed complexes with either streptavidin-chlorin e6 conjugates and biotinylated first specific anti-mOGG1 antibodies or first specific anti-mOGG1 antibodies and chlorin e6-conjugated anti-rabbit IgG secondary antibodies. Similar results were obtained when rose bengal was used as photosensitizer instead of chlorin e6. The rate of the photochemical reaction of mOGG1 site-directed by all three chlorin e6 antibody complexes was not affected by the presence of the singlet oxygen scavenger sodium azide. Site-directed photoactivatable probes having the capacity to generate reactive oxygen species (ROS) while destroying the DNA repair system in malignant cells and tumors may represent a powerful strategy to boost selectivity, penetration and efficacy of current photodynamic (PDT) therapy methodologies.

Keywords: Antibody; PDT; Streptavidin; Avidin; Chlorin e6; Porphyrin; Proteolysis; OGG1; Light

1. Introduction

PDT has been applied to control insects and bacterial infestations but its greatest potential resides in combating human cancers and other diseases. PDT in cancer therapy is based on the observation that certain non-toxic photosensitizer (PS) molecules, of which the most prominent is Photofrin, have a tendency to accumulate preferentially in malignant cells [1,2]. Although most PS molecules with potential applicability for PDT are still in the research phase, PS structurally share the tetrapyrrole nucleus and include porphyrins, chlorins, bacteriochlorins, phthalocyanines and texaphyrins [3]. Other PS molecules with PDT potential under investigation include rose bengal, Toluidine blue, Methylene blue, acridines and perylenequinones such as hypericin [4–7]. PDT involves the systemic administration of PS to patients followed by a few hours of topical irradiation of the tumor area with visible light of the appropriate wavelength to excite the PS to its singlet state, which can react with molecular oxygen and ultimately form ROS. Thus, it is thought that PDT-induced malignant cell death is the result of ROS generation and consequent cell damage.

ROS such as hydroxyl radicals and singlet oxygen are highly reactive and can damage macromolecules including

* Corresponding author. Tel.: +1 631 444 3050; fax: +1 631 444 3218.
E-mail address: conlon@pharm.sunysb.edu (K.A. Conlon).

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DNA. ROS formation also increases when cells are exposed to environmental pollutants [8,9], certain drugs [10], nutrient deprivation [11], oxidizing agents or ionizing radiation [12–14] and during some pathological processes such as inflammation or ischemia-reperfusion [15]. Reaction between ROS and DNA leads to several base modifications including 7,8-dihydro-8-oxoguanine (8-oxoG), a lesion that if not enzymatically repaired may lead to mutations, apoptosis and cell death. The repair of 8-oxoG involves 8-oxoG DNA glycosylase 1 (OGG1), a member of the base excision repair pathway (BER) [16]. The first step of this repair pathway is the recognition and removal of the modified base by a DNA glycosylase, leaving an abasic site. Subsequently, the abasic site is excised, and the repair is completed by a phosphodiesterase, DNA polymerase, and DNA ligase [17]. 8-OxoG repair activities have been reported in mammals including mice [18,19], human leukocytes and HeLa cells [20,21]. Recently, liver cells from homozygous $ogg^{-/-}$ mice have shown a 10-fold increase in G:C to T:A transversion frequencies in their DNA [22,23].

PDT has advantages over cancer radiation therapy and cancer chemotherapy in that it has few side effects and has dual selectivity because PS tend to accumulate in tumors, or other tissue lesions, and visible light sources can be accurately focused largely on the tumor mass. The main disadvantage of current PDT is the lack of strategies that confer controlled site-directed specificity to the PS for the tumor or specific lineage of malignant cells within the tumor. Antibodies have been used extensively and successfully in biomedical research and are the basis of numerous clinical assays largely because of their unique molecular specificity for a given epitope and/or collection of epitopes and for their ability to be modified chemically and site-direct in situ secondary responses involving, for instance, enzymatic or photochemical reactions. In passive cancer immunotherapy, exogenous antibodies directed against antigens expressed in malignant cells are administered systemically to the patient [24,25]. Mouse monoclonal antibodies have been the main source of antibody reagents for this purpose and recently the mouse system has been replaced by mouse-human antibody chimeras and/or fragments thereof to avoid eliciting immune responses against these non-human proteins [26]. This technology has resulted in the growth of antibody-based therapies approved for cancer treatment (for a comprehensive review, see [27]). A major limitation of using mouse antibodies or humanized antibody chimeras for cancer therapy is that they do not kill tumor cells upon binding or by themselves. Thus, much effort has been placed in introducing modifications to antibodies so they are able to carry a toxin or a radioisotope, thereby site-directing the radioisotope or toxin to the antigen site. PS conjugated to antibodies have been used to site-direct PDT to specific tumor cells [28]. Site-directed PDT is being applied to ovarian cancers using the epidermal growth factor receptor (EGFR) as a molecular target to modulate cell proliferation [29–31]. Conjugating PS to antibodies for site-directed PDT still has limitations, however. These limitations are associated with the non-specific uptake of PS-conjugates by cells and the tumor tissue penetration of PS-antibody conjugates [28,32]. Therefore to circumvent these limitations, more research is needed.

Reagents that non-enzymatically digest proteins and nucleic acids are useful tools to obtain functional, sequence and structural information about these macromolecules. Light-induced proteolysis is an attractive alternative to enzymatic and chemically induced protein photolysis. The reactions of photoproteases are easily modulated because they are initiated by the action of light and terminated on its removal. Photoproteases and antibodies may prove to be a powerful combination in the design of new strategies for improving the effectiveness of site-directed PDT [28,33,34].

In this paper we describe strategies to conjugate PS with PDT value onto monospecific antibodies directed against a mammalian OGG1, a major enzyme in the BER pathway. These reagents were capable of photolyzing this enzyme in vitro in the presence of several specific protein probes and visible light of a wavelength commonly used in PDT. We also show that photoproteolysis may not involve a singlet oxygen mechanism. From these results and previous work, the expectation is that these site-directed reagents will improve tumor penetration by digesting the extracellular matrix, as well as to vastly increase malignant cell death by boosting ROS production while simultaneously hindering repair of oxidatively damaged DNA by photoproteolytically inactivating OGG1 and other enzymes of the BER pathway.

2. Experimental

2.1. Materials

Rose bengal and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Chlorin e6 and pyropheophorbide-a were from Frontier Scientific, Inc. (Logan, UT). Coomassie blue, dithiothreitol (DTT), iodoacetamide (IAA), and Tween 20 were purchased from Sigma Chemical Co. (Saint Louis, MO). Dimethyl sulfoxide (DMSO) was purchased from Spectrum Laboratory Products (New Brunswick, NJ). Glycerol and Triton X-100 were purchased from Fisher Scientific Co. (Springfield, NJ). Sodium dodecyl sulfate (SDS) was purchased from British Drug Houses (Poole, England). NHS-LC-biotin and Slide-A-Lyzer Mini-Dialysis Units (10,000 MW cut-off) were purchased from Pierce Biotechnology, Inc. (Rockford, IL). Streptavidin was from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Isolation and purification of mOGG1 were as previously described [18]. All other chemicals were obtained commercially, were of reagent grade, and were used without further purification.