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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

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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

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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 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 manuscript 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.

2.1.1. Antibodies

Rabbit anti-mOGG1 antiserum directed against the whole wild-type recombinant mOGG1 polypeptide was as previously described [35]. Affinity purified goat anti-rabbit IgG (anti-heavy and light chains) antibodies were purchased from Kirkegaard and Perry Laboratories (Gaithersburg, MD).

2.2. Methods

2.2.1. Fractionation and enrichment of rabbit anti-mOGG1 IgG—10 ml of rabbit anti-mOGG1 antiserum was diluted with one volume of ice cold 4M ammonium sulfate buffered solution and immunoglobulins were allowed to precipitate by incubation on ice overnight. The precipitate was pelleted by centrifugation at 10,000 x g for 20 min at 4°C. The pelleted immunoglobulin fraction was resuspended in ice cold PBS (140 mM NaCl, 10 mM phosphate buffer, pH 7.5) using a volume equivalent to that of the pellet. The above centrifugation step was repeated and the final pellet was resuspended in 1 ml of PBS supplemented with 10% (v/v) glycerol and IgG fractions stored at -20°C.

2.2.2. Reaction of chlorin e6 and pyropheophorbide-a with EDC—Chlorin e6 (2 mg, 3.4 µmol) was dissolved in 920 µl of DMSO. To this solution was added 80 µl of DMSO

containing EDC (0.8 mg, 4.2 μ mol). The mixture was incubated at room temperature for 1 h on a Roto-Shake Genie (Scientific Industries, Bohemia, NY), after which, the mixture was stored at -20°C . Pyropheophorbide-a (2 mg, 3.7 μ mol) was dissolved in 910 μ l of DMSO. To this solution was added 90 μ l of DMSO containing EDC (0.9 mg, 4.7 μ mol). The mixture was incubated at room temperature for 1 h on a Roto-Shake Genie (Scientific Industries, Bohemia, NY), after which, the mixture was stored at -20°C .

2.2.3. Conjugation of chlorin e6 and pyropheophorbide-a to rabbit anti-mOGG1 antibodies and affinity purified goat anti-rabbit IgG antibodies—Rabbit anti-mOGG1 antibodies (whole serum or IgG fraction) and affinity purified goat anti-rabbit antibodies were conjugated to an average of 4–6 porphyrin molecules. Briefly, to 200 μ l of anti-mOGG1 antibodies or affinity purified goat anti-rabbit antibodies was added 2 μ l containing chlorin e6-EDC or pyropheophorbide-a-EDC. Alternatively, to 50 μ l of anti-mOGG1 antibodies (IgG fraction) was added 1 μ l containing chlorin e6-EDC or pyropheophorbide-a-EDC. The mixture was incubated at room temperature for 30 min on a Roto-Shake Genie (Scientific Industries, Bohemia, NY). The solution of the conjugate was dialyzed against 100 ml of MSM-Pipes [36] supplemented with 0.2% (w/v) SDS at 4°C overnight.

2.2.4. Conjugation of the N-hydroxysuccinimide (NHS) ester of rose bengal-hexanoic acid (RBHA) to rabbit anti-mOGG1 antibodies and affinity purified goat anti-rabbit IgG antibodies—The synthesis of the NHS ester of RBHA was previously described [37]. Rabbit anti-mOGG1 antibodies and affinity purified goat anti-rabbit IgG antibodies were labeled with rose bengal as described previously [38]. Briefly, to 50 μ l of anti-mOGG1 antibodies (whole serum or IgG fraction) or 100 μ l containing 10 μ g of affinity purified goat anti-rabbit IgG antibodies was added 2 μ g of RBHA-NHS ester (2 mg/ml stock solution in DMSO). The mixtures were incubated at room temperature for 30 min on a Roto-Shake Genie (Scientific Industries, Bohemia, NY). The solutions of the conjugates were dialyzed against 100 ml of MSM-Pipes supplemented with 0.2% (w/v) SDS at 4°C overnight.

2.2.5. Biotinylation of rabbit anti-mOGG1 IgG-enriched fractions—To 100 μ l of anti-mOGG1 antibodies (IgG fraction) was added 4 μ g of NHS-LC-biotin (4 mg/ml stock solution in DMSO). The mixture was incubated at room temperature for 30 min on a Roto-Shake Genie (Scientific Industries, Bohemia, NY). The solution of the conjugate was dialyzed against 100 ml of MSM-Pipes supplemented with 0.2% (w/v) SDS at 4°C overnight.

2.2.6. Conjugation of chlorin e6 to streptavidin—Streptavidin was conjugated to an average of 4–6 chlorin e6 molecules. Briefly, to a 50 μ l solution containing 100 μ g of streptavidin was added 2 μ l of chlorin e6-EDC. The mixture was incubated at room temperature for 30 min on a Roto-Shake Genie (Scientific Industries, Bohemia, NY). The solution of the conjugate was dialyzed against 100 ml of MSM-Pipes supplemented with 0.2% (w/v) SDS at 4°C overnight.

2.2.7. Conjugation of the NHS ester of RBHA to streptavidin—Streptavidin was conjugated to an average of 4–6 rose bengal molecules. Briefly, to a 50 μ l solution containing 100 μ g of streptavidin was added 2 μ g of RBHA-NHS. The mixture was incubated at room temperature for 30 min on a Roto-Shake Genie (Scientific Industries, Bohemia, NY). The solution of the conjugate was dialyzed against 100 ml of MSM-Pipes supplemented with 0.2% (w/v) SDS at 4°C overnight.

2.2.8. Photochemistry using either first specific porphyrin-conjugated antibodies or first specific-porphyrin-conjugated secondary antibody

complexes—Two methods were used to photochemically induce the photolysis of a mOGG1 by porphyrin-conjugated or rose bengal-conjugated antibodies: (A) a direct procedure and (B) an indirect procedure. Direct procedure: Solutions (10 μ l) containing 2 μ g of mOGG1 in MSM-Pipes supplemented with 0.2% (w/v) SDS were placed into clear borosilicate glass tubes. To each solution was added 1 μ l of porphyrin-conjugated or rose bengal-conjugated anti-mOGG1 antibodies (whole serum or IgG fraction) at the appropriate dilution in MSM-Pipes supplemented with 0.2% (w/v) SDS. For control experiments, 1 μ l of unconjugated anti-mOGG1 antibodies at the appropriate dilution in MSM-Pipes supplemented with 0.2% (w/v) SDS was added. All mixtures were incubated at room temperature in the dark for 15 min. Reaction mixtures were irradiated for 30 min at 25°C with visible light from an illumination head connected through an 80 cm long, 7 mm diameter fiber optic to a fan cooled 150 W halogen lamp as previously described [37]. The lamp spectral data is available at www.schott-fostec.com. For reaction mixtures containing rose bengal-conjugated antibodies, irradiations were performed using a 500 nm wavelength cut-off filter (Morrell Instruments, Melville, NY). For reaction mixtures containing porphyrin-conjugated antibodies, irradiations were performed using a 600 nm wavelength cut-off filter (Morrell Instruments, Melville, NY). After irradiation, products were characterized by SDS-PAGE analysis. The calculated fluence at 630 nm for an illumination area of 4.5 cm² was 1.1 μ W/cm² as determined by a LaserCheck power meter (Coherent, Auburn, CA). The fluence rate was calculated to be 2 mJ/cm². SDS-PAGE was as follows: proteins were solubilized in boiling sample loading buffer supplemented with 0.2% (w/v) SDS and incubated for 30 min at room temperature in the dark with 87 mM IAA and 87 mM dibasic sodium phosphate before separation by SDS-PAGE [36]. mOGG1 digests were visualized using Coomassie blue stained SDS-PAGE gels. Gels were stained overnight. Indirect procedure: Solutions (10 μ l) containing 2 μ g of mOGG1 in MSM-Pipes supplemented with 0.2% (w/v) SDS were placed into clear borosilicate glass tubes. To each solution was added 1 μ l of unconjugated anti-mOGG1 antibodies (IgG fraction) at the appropriate dilution in MSM-Pipes supplemented with 0.2% (w/v) SDS, and the mixtures were incubated at room temperature in the dark for 15 min. Thereafter, 1 μ l of a solution containing 100 μ g/ml of porphyrin or rose bengal-conjugated goat anti-rabbit IgG secondary antibodies was added to each tube. Mixtures were incubated in the dark at 37°C for 15 min. Reaction mixtures were irradiated for 30 min at 25°C. After irradiation, proteins were solubilized in SDS, separated by SDS-PAGE and mOGG1 digests visualized by Coomassie blue as described for the direct procedure.

2.2.9. Photochemistry using biotinylated first specific antibodies-porphyrin conjugated streptavidin complexes—Solutions (10 μ l) containing 2 μ g of mOGG1 in MSM-Pipes supplemented with 0.2% (w/v) SDS were placed into clear borosilicate glass tubes. To each solution was added biotinylated anti-mOGG1 antibodies (IgG fraction) at the appropriate dilution in MSM-Pipes supplemented with 0.2% (w/v) SDS, and the mixtures were incubated in the dark for 15 min. Thereafter, 1 μ l of a solution containing chlorin e6 or rose bengal-conjugated streptavidin was added to each tube. For control experiments, 1 μ l containing 2 μ g of unconjugated streptavidin at the appropriate dilution in MSM-Pipes supplemented with 0.2% (w/v) SDS was added. Mixtures were incubated in the dark at 37°C for 15 min. Reaction mixtures were irradiated for 30 min at 25°C. After irradiation, proteins were solubilized in SDS, separated by SDS-PAGE and mOGG1 digests visualized by Coomassie blue as described for the direct procedure.

3. Results

3.1. Light induced photolysis of mogg1 by porphyrin-conjugated anti-mOGG1 antibodies (direct method)

To conjugate the porphyrins, chlorin e6 and pyropheophorbide-a, to anti-mOGG1 antibodies or goat anti-rabbit IgG secondary antibodies, each compound was first reacted with EDC in DMSO at room temperature for 30 min. After reaction, the solutions were either used for antibody conjugation or aliquotted and stored at -20°C .

MOGG1 was reacted directly with either chlorin e6-conjugated anti-mOGG1 antibodies or pyropheophorbide-a-conjugated anti-mOGG1 antibodies (in whole serum) in the presence of visible light of wavelengths greater than 600 nm at 25°C for 30 min (Figure 1). Significant photolysis of mOGG1 was achieved at dilutions of 1:20 and 1:50 for each porphyrin-conjugated antibody. To circumvent problems associated with the impurities of whole serum, an IgG fraction of anti-mOGG1 serum was isolated and conjugated to chlorin-e6, pyropheophorbide-a and rose bengal. Efficient photolysis of mOGG1 was seen at several dilutions of antibody conjugates (Figure 2). No photolysis of mOGG1 was observed when unconjugated anti-mOGG1 antibodies were used in place of porphyrin-conjugated anti-mOGG1 antibodies (data not shown).

3.2. Light induced photolysis of mOGG1 by porphyrin-conjugated anti-rabbit IgG secondary-primary antibody complexes (indirect method)

Porphyrin-conjugated goat anti-rabbit IgG secondary antibodies were tested for their ability to induce the photolysis of mOGG1 in a complex with primary specific anti-mOGG1 antibodies using visible light at wavelengths greater than 600 nm. The reaction was compared to that using porphyrin-conjugated anti-mOGG1 antibodies. Dilutions of anti-mOGG1 antibodies were the same throughout. In these experiments and those that follow, the IgG fraction of anti-mOGG1 antibodies is used as primary specific antibody. For each porphyrin, the rate of photolysis of mOGG1 was faster with the indirect method than with the direct method (Figure 3A). Reaction mixtures incubated in the dark showed no photolysis of mOGG1 (Figure 3B). No photolysis of mOGG1 was observed when unconjugated goat anti-rabbit IgG secondary antibodies were used in place of porphyrin-conjugated goat anti-rabbit IgG secondary antibodies (data not shown).

3.3. Presence of sodium azide does not alter light induced photolysis of porphyrin-conjugated anti-mOGG1 antibodies—Because the mode of action of PDT is commonly thought to occur through generation of singlet oxygen by the excited state of the PS, thus resulting in ROS formation, the photolysis was performed in the presence of sodium azide, a scavenger of singlet oxygen. Sodium azide (0.5 mM or 2 mM) was added to reaction mixtures containing mOGG1-anti-mOGG1 antibody complexes and chlorin e6-conjugated goat anti-rabbit IgG secondary antibodies. Solutions were irradiated at 25°C for 30 min at wavelengths greater than 600 nm. The presence of sodium azide had no effect on the photo-induced degradation of mOGG1 (Figure 4).

3.4 Light induced photolysis of mOGG1 by porphyrin-conjugated streptavidin—Streptavidin was conjugated to chlorin e6-EDC or rose bengal under conditions similar to those used for antibody conjugations. Anti-mOGG1 antibodies were biotinylated in the same manner. The ability of rose bengal-conjugated streptavidin to photolyze mOGG1 in a complex with biotinylated anti-mOGG1 antibodies was evaluated at different concentrations of streptavidin (Figure 5A) and different dilutions of primary specific antibody (Figure 5B). Reactions were performed at 25°C for 30 min with visible light at wavelengths greater than

500 nm. Results from these experiments demonstrated that rose bengal-conjugated streptavidin was able to photodigest mOGG1 under these conditions.

The photoreaction was performed using chlorin e6-conjugated streptavidin and visible light at wavelengths greater than 600 nm. Controls included a reaction mixture containing unconjugated streptavidin and a reaction mixture incubated in the dark. Chlorin e6-conjugated streptavidin induced the photolysis of mOGG1. No reaction of mOGG1 was observed under control conditions (Figure 6).

Discussion

We previously showed that rose bengal-conjugated primary specific antibodies or rose bengal-conjugated-secondary antibodies forming a complex with untagged primary specific antibodies can induce the direct or indirect photolysis respectively of antibody-targeted proteins in the presence of visible light [37]. In addition, using similar strategies, we demonstrated that non-antibody PS-conjugated protein ligands, such as rose bengal conjugated heavy meromyosin specifically photolyzed actin filaments [39]. Experiments with tissue culture cells revealed that site-directed photoproteases are as effective in vitro as in situ [37,39]. Here we describe similar in vitro results to those reported earlier using rose bengal conjugates. Porphyrin-conjugated antibody complexes and porphyrin-conjugated streptavidin biotinylated-antibody complexes irradiated with wavelengths greater than 600 nm, the portion of the visible light spectrum useful for PDT, were capable of photodigesting the purified recombinant mOGG1 protein in vitro. Photoproteolysis of recombinant mOGG1 protein by specific protein-porphyrin conjugates was consistent with in vitro results reported earlier for rose bengal conjugates with specificity for this mammalian DNA repair enzyme.

To conduct porphyrin site-directed protein photolysis experiments described in this paper, we chose the porphyrins chlorin e6 and pyropheophorbide-a, compounds that have a λ_{max} of absorption above 600 nm, have significant molar extinction coefficients at these wavelengths and have been used extensively as PS for PDT studies [40–44]. In control experiments, mOGG1 was efficiently photolyzed by these porphyrins at concentrations ranging from 6.4 μM to 64 μM (data not shown). The bacterially-expressed purified mOGG1 recombinant protein used in these studies was an active enzyme that shares properties and amino acid sequence homology with its authentic human counterpart.

To conjugate porphyrin compounds to antibody fractions or streptavidin, porphyrins were first reacted with EDC. Porphyrin-EDC compounds were then directly conjugated to primary specific antibodies, goat anti-rabbit IgG secondary antibody fractions or streptavidin. When mOGG1 formed an immunocomplex directly with either chlorin e6-conjugated anti-mOGG1 antibodies or pyropheophorbide-a-conjugated anti-mOGG1 antibodies in the presence of visible light, the mOGG1 polypeptide was photolyzed as judged by Coomassie blue staining of SDS-PAGE gels. When these photoreactions were performed using an indirect method combining unconjugated anti-mOGG1 antibodies complexed to chlorin e6-conjugated goat anti-rabbit IgG secondary antibodies or pyropheophorbide-a-conjugated goat anti-rabbit IgG secondary antibodies, the mOGG1 polypeptide was photolyzed to a larger extent than that observed for mOGG1-chlorin e6-conjugated or pyropheophorbide-a-conjugated anti-mOGG1 antibody immunocomplexes. This result may be explained by the local increase of PS concentration at the reaction site brought about by porphyrin-conjugated secondary antibodies-anti-mOGG1 antibody complexes. Furthermore, as a result of possible detrimental effects of the conjugation reaction, porphyrin-conjugated anti-mOGG1 antibodies may be expected to have a lower binding affinity for mOGG1 than unconjugated anti-mOGG1 antibodies, thus making the direct method less efficient than the indirect method.

To determine the optimal reaction conditions for chlorin e6-conjugated streptavidin, photoreactions were performed with rose bengal-conjugated streptavidin and light at wavelengths greater than 500 nm. From these experiments, it was determined that a streptavidin concentration of 1 μ g in conjunction with 1 μ l of biotinylated anti-mOGG1 antibodies gave the largest amount of photoreaction. When the experiment was conducted under these conditions using chlorin e6-conjugated streptavidin, reaction of mOGG1 was less extensive than with either the direct or indirect methods and chlorine e6-conjugated antibodies (compare Fig. 3, lanes 1 and 2 with Fig. 6, lane 2). Possible explanations for this observation may be 1) the biotinylation may be detrimental to the primary antibody, 2) the PS conjugation of streptavidin may decrease its affinity for biotin, 3) the increase of the local concentration of PS-conjugated streptavidin is lower than that found for PS-conjugated secondary antibody complexes, and 4) the smaller size of streptavidin as compared to that of the antibodies may keep the PS at a longer distance from mOGG1, thus allowing for less reaction. Any or all of the above possibilities will contribute to the reduction of the photoreaction efficiency.

Photolysis of mOGG1 was dependent on visible light and was not altered by addition of sodium azide to reaction mixtures, indicating that a singlet oxygen mechanism may not be involved in the in vitro photolysis of the mOGG1 polypeptide by porphyrins. The inability of sodium azide to quench the photoreaction is intriguing because chlorin e6, pyropheophorbide a, and rose bengal produce singlet oxygen upon irradiation. It should be noted that sodium azide had no effect when the reaction was performed with free PS rather than PS bound to antibodies (data not shown). Further investigation will be necessary to elucidate the mechanism of protein photolysis by these PS-antibody conjugates under our experimental conditions.

Results presented here are significant to the clinical applications of PDT in that the specificity of the primary specific antibodies chosen for these studies was directed against mOGG1, a key enzyme of mammalian repair of oxidized DNA. ROS-induced cell damage in general and oxidatively damaged DNA in particular are thought to be the main reasons for malignant cell death and tumor ablation after PDT [1,2,28,45]. Since the mOGG1 protein was successfully photolyzed by porphyrin-conjugated antibodies or porphyrin-conjugated streptavidin when irradiated with wavelengths commonly used for PDT, the success of this strategy opens the possibility of using porphyrin-conjugated probes to improve site-directed PDT both by in situ producing ROS in tumor cells and site-directing part of the photochemical reaction to obliterate an important DNA repair enzyme, OGG1. In this manner, malignant cells will be overwhelmed by the generation of ROS and have a decreased capacity to combat such highly reactive byproducts of the site-directed photochemical reaction. Furthermore, minimal modifications to strategies presented here, including the use of combinations of specific antibodies directed against a collection of DNA repair enzymes and the encapsulation of these PS reagents into stabilized liposomes, will further increase the half life of PS-conjugated reagents, tumor accessibility and malignant cell lethality, thereby, boosting the overall effectiveness of this site-directed PDT methodology for cancer therapy.

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References

1. Berg K, Selbo PK, Weyergang A, Dietze A, Prasmickaite L, Bonsted A, Engesaeter BO, Angell-Petersen E, Warloe T, Frandsen N, Hogset A. Porphyrin-related photosensitizers for cancer imaging and therapeutic applications. *J Microsc* 2005;218:133–147. [PubMed: 15857375]
2. Henderson BW, Dougherty TJ. How does photodynamic therapy work? *Photochem Photobiol* 1992;55:145–157. [PubMed: 1603846]

3. Harris F, Sayed Z, Hussain S, Phoenix D. An investigation into the potential of phenothiazinium-based photo-sensitisers to act as PDT agents. *Photodiagn and Photodyn Ther* 2004;1:231–239.
4. Bhatti M, MacRobert A, Meghji S, Henderson B, Wilson M. A study of the uptake of toluidine blue O by *Porphyromonas gingivalis* and the mechanism of lethal photosensitization. *Photochem Photobiol* 1998;68:370–376. [PubMed: 9747591]
5. Hass BS, Webb RB. Photodynamic effects of dyes on bacteria. IV. Lethal effects of acridine orange and 460- or 500-nm monochromatic light in strains of *Escherichia coli* that differ in repair capability. *Mutat Res* 1981;81:277–285. [PubMed: 7029255]
6. Kubin A, Wierrani F, Jindra RH, Loew HG, Grunberger W, Ebermann R, Alth G. Antagonistic effects of combination photosensitization by hypericin, meso-tetrahydroxyphenylchlorin (mTHPC) and photofrin II on *Staphylococcus aureus*. *Drugs Exp Clin Res* 1999;25:13–21. [PubMed: 10337500]
7. Schafer M, Schmitz C, Facius R, Horneck G, Milow B, Funken KH, Ortner J. Systematic study of parameters influencing the action of Rose Bengal with visible light on bacterial cells: comparison between the biological effect and singlet-oxygen production. *Photochem Photobiol* 2000;71:514–523. [PubMed: 10818781]
8. Kim HN, Morimoto Y, Tsuda T, Ootsuyama Y, Hirohashi M, Hirano T, Tanaka I, Lim Y, Yun IG, Kasai H. Changes in DNA 8-hydroxyguanine levels, 8-hydroxyguanine repair activity, and hOGG1 and hMTH1 mRNA expression in human lung alveolar epithelial cells induced by crocidolite asbestos. *Carcinogenesis* 2001;22:265–269. [PubMed: 11181447]
9. Tsurudome Y, Hirano T, Yamato H, Tanaka I, Sagai M, Hirano H, Nagata N, Itoh H, Kasai H. Changes in levels of 8-hydroxyguanine in DNA, its repair and OGG1 mRNA in rat lungs after intratracheal administration of diesel exhaust particles. *Carcinogenesis* 1999;20:1573–1576. [PubMed: 10426809]
10. Kinoshita A, Wanibuchi H, Imaoka S, Ogawa M, Masuda C, Morimura K, Funae Y, Fukushima S. Formation of 8-hydroxydeoxyguanosine and cell-cycle arrest in the rat liver via generation of oxidative stress by phenobarbital: association with expression profiles of p21(WAF1/Cip1), cyclin D1 and Ogg1. *Carcinogenesis* 2002;23:341–349. [PubMed: 11872643]
11. Conlon KA, Grollman AP, Berrios M. Immunolocalization of 8-oxoguanine in nutrient-deprived mammalian tissue culture cells. *J Histotech* 2000;23:37–44.
12. Kasai H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, Tanooka H. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* 1986;7:1849–1851. [PubMed: 3769133]
13. Kasai H, Tanooka H, Nishimura S. Formation of 8-hydroxyguanine residues in DNA by X-irradiation. *Gann* 1984;75:1037–1039. [PubMed: 6526216]
14. Ravanat JL, Cadet J. Reaction of singlet oxygen with 2'-deoxyguanosine and DNA. Isolation and characterization of the main oxidation products. *Chem Res Toxicol* 1995;8:379–388. [PubMed: 7578924]
15. Tsuruya K, Furuichi M, Tominaga Y, Shinozaki M, Tokumoto M, Yoshimitsu T, Fukuda K, Kanai H, Hirakata H, Iida M, Nakabeppu Y. Accumulation of 8-oxoguanine in the cellular DNA and the alteration of the OGG1 expression during ischemia-reperfusion injury in the rat kidney. *DNA Repair (Amst)* 2003;2:211–229. [PubMed: 12531391]
16. Demple B, Harrison L. Repair of oxidative damage to DNA: enzymology and biology. *Annu Rev Biochem* 1994;63:915–948. [PubMed: 7979257]
17. Lindahl T. Recognition and processing of damaged DNA. *J Cell Sci Suppl* 1995;19:73–77. [PubMed: 8655650]
18. Rosenquist TA, Zharkov DO, Grollman AP. Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. *Proc Natl Acad Sci USA* 1997;94:7429–7434. [PubMed: 9207108]
19. Yamamoto F, Kasai H, Bessho T, Chung MH, Inoue H, Ohtsuka E, Hori T, Nishimura S. Ubiquitous presence in mammalian cells of enzymatic activity specifically cleaving 8-hydroxyguanine-containing DNA. *Jpn J Cancer Res* 1992;83:351–357. [PubMed: 1506269]
20. Bessho T, Tano K, Kasai H, Ohtsuka E, Nishimura S. Evidence for two DNA repair enzymes for 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in human cells. *J Biol Chem* 1993;268:19416–19421. [PubMed: 8366089]

21. Chung MH, Kim HS, Ohtsuka E, Kasai H, Yamamoto F, Nishimura S. An endonuclease activity in human polymorphonuclear neutrophils that removes 8-hydroxyguanine residues from DNA. *Biochem Biophys Res Commun* 1991;178:1472–1478. [PubMed: 1872860]
22. Klungland A, Rosewell I, Hollenbach S, Larsen E, Daly G, Epe B, Seeberg E, Lindahl T, Barnes DE. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc Natl Acad Sci USA* 1999;96:13300–13305. [PubMed: 10557315]
23. Minowa O, Arai T, Hirano M, Monden Y, Nakai S, Fukuda M, Itoh M, Takano H, Hippou Y, Aburatani H, Masumura K, Nohmi T, Nishimura S, Noda T. Mmh/Ogg1 gene inactivation results in accumulation of 8-hydroxyguanine in mice. *Proc Natl Acad Sci USA* 2000;97:4156–4161. [PubMed: 10725358]
24. Del Governatore M, Hamblin MR, Shea CR, Rizvi I, Molpus KG, Tanabe KK, Hasan T. Experimental photoimmunotherapy of hepatic metastases of colorectal cancer with a 17.1A chlorin(e6) immunoconjugate. *Cancer Res* 2000;60:4200–4205. [PubMed: 10945630]
25. Riggs MW, Schaefer DA, Kapil SJ, Barley-Maloney L, Perryman LE. Efficacy of monoclonal antibodies against defined antigens for passive immunotherapy of chronic gastrointestinal cryptosporidiosis. *Antimicrob Agents Chemother* 2002;46:275–282. [PubMed: 11796330]
26. van Dongen GA, Visser GW, Vrouwenraets MB. Photosensitizer-antibody conjugates for detection and therapy of cancer. *Adv Drug Deliv Rev* 2004;56:31–52. [PubMed: 14706444]
27. Sharkey RM, Goldenberg DM. Targeted therapy of cancer: new prospects for antibodies and immunoconjugates. *CA Cancer J Clin* 2006;56:226–243. [PubMed: 16870998]
28. Hamblin M, Bamberg M, Miller J, Hasan T. Cationic photoimmunoconjugates between monoclonal antibodies and hematoporphyrin: selective photodestruction of ovarian cancer cells. *Appl Opt* 1998;37:7184–7192.
29. Cengel KA, Hahn SM, Glatstein E. C225 and PDT combination therapy for ovarian cancer: the play's the thing. *J Natl Cancer Inst* 2005;97:1488–1489. [PubMed: 16234556]
30. del Carmen MG, Rizvi I, Chang Y, Moor AC, Oliva E, Sherwood M, Pogue B, Hasan T. Synergism of epidermal growth factor receptor-targeted immunotherapy with photodynamic treatment of ovarian cancer in vivo. *J Natl Cancer Inst* 2005;97:1516–1524. [PubMed: 16234565]
31. Savellano MD, Hasan T. Photochemical targeting of epidermal growth factor receptor: a mechanistic study. *Clin Cancer Res* 2005;11:1658–1668. [PubMed: 15746071]
32. Jain RK. Physiological barriers to delivery of monoclonal antibodies and other macromolecules in tumors. *Cancer Res* 1990;50:814s–819s. [PubMed: 2404582]
33. Jain RK. Tumor physiology and antibody delivery. *Front Radiat Ther Oncol* 1990;24:32–46. 64–38. [PubMed: 2187763]
34. Konan YN, Gurny R, Allemann E. State of the art in the delivery of photosensitizers for photodynamic therapy. *J Photochem Photobiol* 2002;B 66:89–106.
35. Conlon KA, Zharkov DO, Berrios M. Immunofluorescent localization of the murine 8-oxoguanine DNA glycosylase (mOGG1) in cells growing under normal and nutrient deprivation conditions. *DNA Repair (Amst)* 2003;2:1337–1352. [PubMed: 14642563]
36. Berrios M, Fisher PA. A myosin heavy-chain-like polypeptide is associated with the nuclear envelope in higher eukaryotic cells. *J Cell Biol* 1986;103:711–724. [PubMed: 2943745]
37. Conlon KA, Berrios M. Light-induced proteolysis of myosin heavy chain by Rose Bengal-conjugated antibody complexes. *J Photochem Photobiol* 2001;B 65:22–28.
38. Conlon KA, Zharkov DO, Berrios M. Cell cycle regulation of the murine 8-oxoguanine DNA glycosylase (mOGG1): mOGG1 associates with microtubules during interphase and mitosis. *DNA Repair (Amst)* 2004;3:1601–1615. [PubMed: 15474421]
39. Conlon KA, Rosenquist T, Berrios M. Site-directed photochemical disruption of the actin cytoskeleton by actin-binding Rose Bengal-conjugates. *J Photochem Photobiol* 2002;B 68:140–146.
40. Cunderlikova B, Gangeskar L, Moan J. Acid-base properties of chlorin e6: relation to cellular uptake. *J Photochem Photobiol* 1999;B 53:81–90.
41. Delanaye L, Bahri MA, Tfibel F, Fontaine-Aupart MP, Mouithys-Mickalad A, Heine B, Piette J, Hoebeke M. Physical and chemical properties of pyropheophorbide-a methyl ester in ethanol, phosphate buffer and aqueous dispersion of small unilamellar dimyristoyl-l-alpha-phosphatidylcholine vesicles. *Photochem Photobiol Sci* 2006;5:317–325. [PubMed: 16520867]

42. Fernandez JM, Bilgin MD, Grossweiner LI. Singlet oxygen generation by photodynamic agents. *J Photochem Photobiol* 1997;B 37:131–140.
43. Sun X, Leung WN. Photodynamic therapy with pyropheophorbide-a methyl ester in human lung carcinoma cancer cell: efficacy, localization and apoptosis. *Photochem Photobiol* 2002;75:644–651. [PubMed: 12081327]
44. Zenkevich E, Sagun E, Knyukshto V, Shulga A, Mironov A, Efremova O, Bonnett R, Songca SP, Kassem M. Photophysical and photochemical properties of potential porphyrin and chlorin photosensitizers for PDT. *J Photochem Photobiol* 1996;B 33:171–180.
45. Ochsner M. Photodynamic therapy: the clinical perspective. Review on applications for control of diverse tumorous and non-tumorous diseases. *Arzneimittelforschung* 1997;47:1185–1194. [PubMed: 9428971]

Abbreviations

8-oxoG	7, 8-dihydro-8-oxoguanine
BER	base excision repair
DMSO	dimethyl sulfoxide
DTT	dithiothreitol
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EGFR	epidermal growth factor receptor
IAA	iodoacetamide
mOGG1	murine 8-oxoguanine glycosylase 1
NHS	N-hydroxysuccinimide
PDT	photodynamic therapy
PS	photosensitizer
RBHA	rose bengal hexanoic acid
ROS	reactive oxygen species

Figure 1. Photolysis of mOGG1 by first specific porphyrin-conjugated anti-mOGG1 antiserum (direct method)

Recombinant purified mOGG1 was reacted with either chlorin e6-conjugated anti-mOGG1 antibodies or pyropheophorbide-a-conjugated anti-mOGG1 antibodies in the presence of visible light as described in Materials and Methods. All irradiations were conducted at 25°C for 30 min. Each lane of a 7.5–15% (w/v) polyacrylamide gel was loaded with 10 µl of reaction mixture. Gels were stained overnight with Coomassie blue. Lane 1, mOGG1 photolyzed with chlorin e6-conjugated anti-mOGG1 antibodies diluted 1:20. Lane 2, mOGG1 photolyzed with chlorin e6-conjugated anti-mOGG1 antibodies diluted 1:50. Lane 3, mOGG1 photolyzed with pyropheophorbide-a-conjugated anti-mOGG1 antibodies diluted 1:20. Lane 4, mOGG1 photolyzed with pyropheophorbide-a-conjugated anti-mOGG1 antibodies diluted 1:50. Lane 5, unphotolyzed mOGG1 (Molecular mass/Gel migration standard).

Figure 2. Photolysis of mOGG1 by anti-mOGG1 first specific rabbit IgG fractions either conjugated to porphyrin or rose bengal (direct method)

mOGG1 was reacted with chlorin e6, pyropheophorbide-a or rose bengal-conjugated anti-mOGG1 rabbit IgG fractions in the presence of visible light as described in Materials and Methods. All irradiations were conducted at 25°C for 30 min. Each lane of a 7.5–15% (w/v) polyacrylamide gel was loaded with 10 µl of reaction mixture. Gels were stained overnight with Coomassie blue. (A) Lane 1, mOGG1 photolyzed with chlorin e6-conjugated anti-mOGG1 antibodies diluted 1:100. Lane 2, mOGG1 photolyzed with chlorin e6-conjugated anti-mOGG1 antibodies diluted 1:1000. Lane 3, mOGG1 photolyzed with rose bengal-conjugated anti-mOGG1 antibodies diluted 1:100. Lane 4, mOGG1 photolyzed with rose bengal-conjugated anti-mOGG1 antibodies diluted 1:1,000. (B) Lane 1, mOGG1 photolyzed with pyropheophorbide-a-conjugated anti-mOGG1 antibodies diluted 1:10. Lane 2, mOGG1 photolyzed with pyropheophorbide-a-conjugated anti-mOGG1 antibodies diluted 1:100. Lane 3, mOGG1 photolyzed with pyropheophorbide-a-conjugated anti-mOGG1 antibodies diluted 1:1,000. Lane 4, mOGG1 photolyzed with pyropheophorbide-a-conjugated anti-mOGG1 antibodies diluted 1:10,000. (A, B) Lane 5, unphotolyzed mOGG1 (Molecular mass/Gel migration standard).

Figure 3. Photolysis of mOGG1 by either first specific anti-mOGG1 rabbit IgG fractions or anti-rabbit IgG secondary antibodies conjugated to porphyrins

mOGG1 was incubated with chlorin e6 or pyropheophorbide-a-conjugated anti-mOGG1 rabbit IgG fractions (direct method) or with a mixture containing unconjugated anti-mOGG1 rabbit IgG fractions and either chlorin e6 or pyropheophorbide-a-conjugated goat anti-rabbit IgG secondary antibodies (indirect method) in the presence or absence of visible light as described in Materials and Methods. All irradiations were conducted at 25°C for 30 min. Each lane of a 7.5–15% (w/v) polyacrylamide gel was loaded with 10 µl of reaction mixture. Gels were stained overnight with Coomassie blue. (A) Lane 1, mOGG1 photolyzed with 1 µl chlorin e6-conjugated anti-mOGG1 antibodies. Lane 2, mOGG1 photolyzed with 1 µl unconjugated anti-mOGG1 antibodies diluted 1:50 and 1 µl chlorin e6-conjugated secondary antibodies. Lane 3, mOGG1 photolyzed with 1 µl pyropheophorbide-a-conjugated anti-mOGG1 antibodies. Lane 4, mOGG1 photolyzed with 1 µl unconjugated anti-mOGG1 antibodies diluted 1:50 and 1 µl pyropheophorbide-a-conjugated secondary antibodies. (B) Lanes 1–4 are as in A, unirradiated (dark controls). (A,B) Lane 5, unphotolyzed mOGG1 (Molecular mass/Gel migration standard).

Figure 4. Photolysis of mOGG1 by chlorin e6-conjugated secondary antibodies (indirect method) in the presence and absence of sodium azide

mOGG1 was incubated with a mixture containing unconjugated anti-mOGG1 rabbit IgG fractions and chlorin e6-conjugated goat anti-rabbit IgG secondary antibodies in the presence or absence of sodium azide in the presence of visible light as described in Materials and Methods. All irradiations were conducted at 25°C for 30 min. Each lane of a 7.5–15% (w/v) polyacrylamide gel was loaded with 10 μ l of reaction mixture. Gels were stained overnight with Coomassie blue. First specific anti-mOGG1 rabbit IgG fractions were diluted 1:500 and anti-rabbit IgG secondary antibodies were diluted 1:10. Lane 1, mOGG1 photolyzed with unconjugated anti-mOGG1 antibodies and chlorin e6-conjugated secondary antibodies. Lane 2, mOGG1 photolyzed with unconjugated anti-mOGG1 antibodies and chlorin e6-conjugated secondary antibodies in the presence of 0.5 mM sodium azide. Lane 3, mOGG1 photolyzed with unconjugated anti-mOGG1 antibodies and chlorin e6-conjugated secondary antibodies in the presence of 2 mM sodium azide. Lane 4, unphotolyzed mOGG1 (Molecular mass/Gel migration standard).

Figure 5. Photolysis of mOGG1 by biotinylated anti-mOGG1 antibodies-rose bengal-conjugated streptavidin complexes

mOGG1 was incubated with biotinylated anti-mOGG1 rabbit IgG fractions and either unconjugated streptavidin or rose bengal-conjugated streptavidin in the presence of visible light as described in Materials and Methods. All irradiations were conducted at 25°C for 30 min. Each lane of a 7.5–15% (w/v) polyacrylamide gel was loaded with 10 µl of reaction mixture. Gels were stained overnight with Coomassie blue. (A) Lane 1, mOGG1 photolyzed with biotinylated anti-mOGG1 antibodies and 2 µg of unconjugated streptavidin. Lane 2, mOGG1 photolyzed with biotinylated anti-mOGG1 antibodies and 0.5 µg of rose bengal conjugated-streptavidin. Lane 3, mOGG1 photolyzed with biotinylated anti-mOGG1 antibodies and 1 µg of rose bengal conjugated-streptavidin. Lane 4, mOGG1 photolyzed with biotinylated anti-mOGG1 antibodies and 2.0 µg of rose bengal conjugated-streptavidin. Lanes 1–4, biotinylated anti-mOGG1 antibodies diluted 1:50. (B) Lane 1, mOGG1 photolyzed with 1 µl biotinylated anti-mOGG1 antibodies and rose bengal-conjugated streptavidin. Lane 2, mOGG1 photolyzed with biotinylated anti-mOGG1 antibodies diluted 1:2 and rose bengal-conjugated streptavidin. Lane 3, mOGG1 photolyzed with biotinylated anti-mOGG1 antibodies diluted 1:5 and rose bengal-conjugated streptavidin. Lane 4, mOGG1 photolyzed with biotinylated anti-mOGG1 antibodies diluted 1:10 and rose bengal-conjugated streptavidin. (B) Lanes 1–4 contain 1 µg of rose bengal-conjugated streptavidin. Lane 5, unphotolyzed mOGG1 (Molecular mass/Gel migration standard).

Figure 6. Photoproteolysis of mOGG1 by biotinylated anti-mOGG1 antibodies-chlorin e6-conjugated streptavidin complexes

mOGG1 was incubated with 1 μ l biotinylated anti-mOGG1 rabbit IgG fractions and either unconjugated streptavidin or chlorin e6-conjugated streptavidin in the presence or absence of visible light as described in Materials and Methods. All irradiations were conducted at 25°C for 30 min. Each lane of a 7.5–15% (w/v) polyacrylamide gel was loaded with 10 μ l of reaction mixture. Gels were stained overnight with Coomassie blue. Lane 1, mOGG1 photolyzed with biotinylated anti-mOGG1 antibodies and 1 μ g unconjugated streptavidin. Lane 2, mOGG1 photolyzed with biotinylated anti-mOGG1 antibodies and 1 μ g chlorin e6-conjugated streptavidin. Lane 3, mOGG1 incubated in the dark with biotinylated anti-mOGG1 antibodies and 1 μ g chlorin e6-conjugated streptavidin. Lane 4, unphotolyzed mOGG1 (Molecular mass/ Gel migration standard).