Published in final edited form as:

Am J Surg. 2005 March ; 189(3): 302–305. doi:10.1016/j.amjsurg.2004.11.016.

Killing tumor cells: the effect of photodynamic therapy using mono-L-aspartyl chlorine and NS-398

Elizabeth H. Harvey, M.D.^a, John Webber, M.D.^{a,*}, David Kessel, Ph.D.^b, and David Fromm, M.D.^a

^aDepartment of Surgery, Wayne State University School of Medicine, 6C-UHC, 4201 St. Antoine, Detroit, MI 48201, USA

^bDepartment of Pharmacology, Wayne State University School of Medicine, Detroit, MI, USA

Abstract

Background—Photodynamic therapy (PDT) is a useful treatment for malignant tumors. PDT involves the administration of a photosensitive drug that is selected by neoplastic tissues and their vasculature. One such photosensitizer is mono-L-aspartyl chlorine e6 (NPe6). Recent evidence suggests that the presence of the cyclooxygenase-2 (COX-2) inhibitor NS-398 may potentiate the effect of photosensitizing agents. This study was designed to determine if the addition of NS-398 to NPe6-induced PDT in single or fractionated dosing would result in greater tumor kill.

Methods—Colon-38 tumor was subcutaneously implanted into both flanks of mice and allowed to grow to 0.5 to 1.0 cm. Mice were randomly allocated to 5 groups: (1) single dose of NPe6; (2) fractionated dose of NPe6; (3) NS-398 only; (4) single dose of NPe6 + NS-398; and (5) fractionated dose of NPe6 + NS-398. The left flank was shielded from exposure to irradiation. Tumor size was measured before initiation of PDT and at the time of sacrifice.

Results—The initial tumor weights of both flanks were not significantly different between all groups. Tumor weights at the time of death after PDT using NPe6 were significantly less than their paired tumors in the untreated flanks ($P < 0.0001$). Tumor weights in the treated flanks were significantly less in the group receiving the fractionated dosing of NPe6 as compared to the single dose of NPe6 ($P = 0.0037$). NS-398 plus the single dose of NPe6 significantly decreased tumor weight in the PDT-treated flank ($P = 0.035$) at a level equivalent to that observed with fractionated dosing of the photosensitizer in the absence of NS-398. NS-398 did not significantly further decrease tumor weight in the group that received the fractionated dose of NPe6.

Conclusions—Fractionated dosing of NPe6 demonstrated the best tumor kill. However, NS-398 did not potentiate the effect of PDT using fractionated dosing of NPe6. While PDT using the single NPe6 dose significantly decreased tumor weight, the addition of NS-398 potentiated the killing effect.

Keywords

NPe6; COX-2 inhibitor; Photodynamic therapy; Fractionated dosing

*Corresponding author. Tel.: +1-313-745-8775; fax: +1-313-745-1873. webberheather@hotmail.com.

Presented at the 47th Annual Meeting of the Midwest Surgical Association, Mackinac Island, Michigan, August 15–18, 2004

Photodynamic therapy (PDT) can be effective in the treatment of a variety of malignant tumors and initially involves the administration of a photosensitive drug that is accumulated by neoplastic tissue and its vasculature. Subsequent activation of the drug by a wavelength of light corresponding to one of the drug's absorbance bands results in the production of singlet oxygen, which is followed by a localized cytotoxic effect [1]. A limiting step in the application of PDT to malignant tumors relates to the depth of light penetration into the target tissue. Two fairly commonly used drugs in PDT are the photoreactive agent Photofrin (Axcan, Toronto, Canada) and the "pro-drug" aminolevulinic acid (ALA), which is converted by malignant tissue to the photosensitizer protoporphyrin IX. A limitation of both drugs is the wavelength of light required for activation, 630 to 633 nm, which has limited depth of penetration into tissue. A newer photosensitizer that is activated at a longer wavelength is mono-*L*-aspartyl chlorin e6 (NPe6). NPe6 becomes activated at 664 nm, which increases the depth of light penetration by 20% over Photofrin II [1]. This enhanced penetration affords greater tumor kill.

The pharmacokinetics of the photosensitizer also plays a role in the extent of tumor kill. The effectiveness of NPe6 has been related to plasma level of the drug, which peaks within 1 hour of administration [2]. An additional factor may also involve the progressive uptake of NPe6 by the target cells over several hours [3]. We have recently shown that a fractionated dose of NPe6 results in higher plasma concentrations and significantly greater tumor kill than a single dose [4]. However, high plasma concentrations of phototherapeutic agents are associated with toxicity, and it would be desirable to potentiate the effects at lower concentrations.

Cyclooxygenase (COX)-2 inhibitors have recently been shown to potentiate the effects of photosensitization [5]. The purpose of this study was to investigate the effects of COX-2 inhibition during PDT using a single and fractionated dosing scheme of NPe6.

Materials and Methods

This study was approved by the Wayne State University Animal Investigation Committee.

Animals and photosensitizer

Female C57BL/6NCr mice (6–7 weeks old, 20–25 g; Harlan Sprague Dawley, Indianapolis, IN) were subcutaneously implanted with colon-38 (National Cancer Institute, Frederick Cancer Research and Development Center, DCT Tumor Repository, Frederick, MD) in both flanks. The tumors were allowed to grow to 0.5 to 1.0 cm, and the mice were then randomly allocated into 5 groups. The first group ($n = 8$) received a single dose of NPe6 (Light Sciences Corp., Snoqualmie, WA), 5 mg/kg intraperitoneally (IP). The second group ($n = 8$) was given 2 doses (fractionated) of NPe6, 5 mg/kg IP each (total of 10 mg/kg), 24 hours apart. The third group of tumor-bearing mice ($n = 8$) received only the COX-2 inhibitor NS-398 at a dose of 10 mg/kg using the following dosing scheme: at time points 0, 4 hours, 24 hours, 48 hours, and every other day until sacrifice. The fourth group ($n = 8$) received a single dose of NPe6, 5 mg/kg IP, as well as NS-398, using the dosing regimen described above. The fifth group ($n = 8$) was given the fractionated dose of NPe6 (5 mg/kg IP twice

for a total of 10 mg/kg) with NS-398 in the same dosing regimen as described above. PDT was administered to the tumor in the right flank (the left serving as control) 1 hour after the last dose of NPe6 or, in the case of those animals receiving NS-398 only, at a time point equivalent to 1 hour after the last dose of NPe6.

NPe6 was dissolved in sterile water immediately before use and protected from stray light. The mice were kept in darkened cages after NPe6 dosing until the time of irradiation. For irradiation, the mice were anesthetized with sodium pentobarbital, 0.75 mg, and bilateral flaps were created through a mid-line incision to expose the subcutaneous tumor implants. Irradiation was performed 1 hour after the last NPe6 injection had been given. The light dose was always applied to the right flank using a microdiode array (Oncolux System, Light Sciences Corp.) placed 1 cm anterior to the tumor mass using a fluence rate of 9 mW/cm² and a fluence of 61 J/cm² at 664 nm. Tumor in the opposite (left) flank served as a control and was shielded during irradiation with aluminum foil to prevent inadvertent light exposure. After application of PDT, the incision was closed with surgical sutures, and the first dose of NS-398 was given in those groups that required it. The mice were then kept in darkened cages for 3 to 4 weeks. Only those mice that were on the COX-2 dosing regimen were taken out of the cages for IP injections. These injections were performed in subdued lighting to minimize any photosensitivity that the mice may have. At the time of sufficient tumor growth and just prior to PDT the tumors were measured in 2 dimensions using an electronic caliper (VWR International, South Plainfield, NJ). The tumors were measured using the same method at the time of sacrifice. This measurement was converted to weight by the following formula [6]: (length × width²)/2.

Statistics

Results are expressed as the mean ± SD. Statistical analysis was performed using a paired and unpaired *t* test as well as 1-way and 2-way analyses of variance as appropriate comparing treatment groups to controls or to each other. A value of *P* < 0.05 was considered significant.

Results

At the time of randomization of mice to 1 of the 5 treatment groups, the tumor weights of all groups were not significantly different (*P* = 0.07). PDT using the fractionated dose of NPe6 in the absence of COX-2 inhibition was more effective in causing tumor kill than the single dose of the drug (Fig. 1). While we have reported the same finding previously [4], these experiments were repeated for the present study in order to serve as controls for COX-2 inhibition studies.

Tumors exposed to COX-2 inhibition only were not significantly affected by exposure to light (Fig. 2). However, COX-2 inhibition significantly increased the effectiveness of PDT using the single dose of NPe6, which was statistically equivalent to that observed for tumors exposed to the fractionated dose of NPe6 (Fig. 3).

Comments

NPe6 (also known as MACE, ME2906, or LS11) is a pure monomeric compound derived from chlorophyll. Several properties of NPe6 make it a desirable as a photosensitizer for photodynamic therapy. PDT efficacy can be limited by the wavelength of light required to activate a photosensitizer, resulting in decreased depth of tumor kill with shorter wavelength photosensitizers. NPe6 has a strong absorption band at a longer wavelength of light, 664 nm, than the current Food and Drug Administration–approved photosensitizers such as Photofrin (633 nm) and ALA (630 nm). NPe6, therefore, will become activated at a greater depth, providing better tumoricidal effects [1]. NPe6 also has a long triplet state, which is efficiently transferred to molecular oxygen, resulting in a good yield of singlet oxygen [7]. The singlet oxygen rapidly reacts with biomolecules, including phospholipids, cholesterol, and membrane proteins, which can result in reactions that include direct cell damage, cytokine release, immune response activation, and vascular damage [1].

Another factor that can limit the efficacy of PDT is the target tissue concentration of the photosensitizer. A weaker response occurs at low drug concentrations because photosensitizers are degraded (bleached) by light. Thus, an effective concentration of the drug results in a sufficiently activated amount after bleaching that will permit interaction with molecular oxygen. The photodegradation, or bleaching, of NPe6 is more rapid and more complete than certain other photosensitizers (e.g., Photofrin or chloroaluminum sulfonated phthalocyanine), and this photodegradation appears to be irreversible [8]. To avoid toxicity in a photosensitizer, it would be desirable to potentiate a low dose of the drug.

Similar to our previous study, fractionated dosing (5 mg/kg given twice for a total of 10 mg/kg) of NPe6 resulted in significantly greater tumor kill [4]. In the present study, COX-2 inhibition resulted in a decrease in tumor weight with the single dosing regimen of NPe6 (5 mg/kg) and subsequent application of light that was equivalent to that observed with the fractionated dose of NPe6. However, there was no significant further reduction in tumor weight with fractionated dosing of NPe6 (5 mg/kg given twice) associated with the addition of the COX-2 inhibitor.

PDT has been shown to stimulate the release of prostaglandin E₂ from macrophages and fibrosarcoma cells grown in culture. COX is the rate-limiting enzyme in the conversion of arachidonic acid to prostaglandin. PDT induces COX-2 expression [5], which would decrease the efficacy of PDT in tumor killing. In fact, COX-2 has been shown to promote cell growth and to inhibit apoptosis, a known mechanism of tumor killing in PDT. COX-2 overexpression can even result in tumorigenesis [9] and is associated with activation of angiogenic factors [5,9]. Thus, COX-2 inhibition can prohibit further tumor growth and may act to enhance the tumoricidal effects of PDT. On the one hand, one might expect an effect of COX-2 inhibition to augment tumor kill irrespective of a dose of photosensitizer that results in tumor kill because of the negative effect of COX-2 inhibition on angiogenesis. On the other hand, the effectiveness of PDT relies on an intact vascular supply so that sufficient oxygen is present for a cytotoxic effect. Thus, a possible explanation for the effect of COX-2 inhibition using the single dose NPe6 may be due to the single dose of NPe6 not reaching a maximum level for tumor kill and allowing the anti-angiogenic properties of a COX-2

inhibitor to potentiate the reaction. The lack of an effect of COX-2 inhibition on tumor kill using the fractionated dosing regimen of NPe6 for PDT is not clear, but this possibly is related to the fractionated dose resulting in high enough concentrations of activated photosensitizer that no further effect is possible.

In conclusion, fractionated dosing of NPe6-induced PDT demonstrated the greatest amount of tumor kill. Single dosing of NPe6, when combined with a COX-2 inhibitor, was nearly as effective. COX-2 inhibition may be an important adjunct to PDT with NPe6 because a lesser dose of the photosensitizer is necessary for effective tumor kill and thereby may limit toxicity of the photosensitizing drug.

References

1. Chen J, Keltner L, Christophersen J, et al. New technology for deep light distribution in tissue for phototherapy. *Cancer J*. 2002; 8:154–163. [PubMed: 11999949]
2. Kessel D. Determinants of photosensitization by mono-l-aspartyl chlorin e₆. *Photochem Photobiol*. 1989; 49:447–452. [PubMed: 2727084]
3. Roberts WG, Berns MW. In-vitro photosensitization. I. Cellular uptake and subcellular localization of mono-l-aspartyl chlorin e₆, chloroaluminum sulfonated phthaloxyanine and photofrin II. *Lasers Surg Med*. 1989; 9:90–101. [PubMed: 2523995]
4. Webber J, Leeson B, Fromm D, Kessel D. Effects of photodynamic therapy using fractionated dosing of mono-l-aspartyl chlorin e₆ in a mouse tumor model. *J Photochem Photobiol B Biol*. 2005; 78:135–140.
5. Ferrario A, von Tiehl K, Wong S, et al. Cyclooxygenase-2 inhibitor treatment enhances photodynamic therapy-mediated tumor response. *Cancer Res*. 2002; 62:3956–3961. [PubMed: 12124326]
6. Valeriote, FA.; Corbett, TH.; Baker, LH., editors. *Proceedings of the 22nd Annual Cancer Symposium*, Detroit, Michigan. Boston, MA: Kluwer Academic Publishers; 1992. Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development; p. 75
7. Spikes JD, Bommer JC. Photosensitizing properties of mono-l-aspartyl chlorin e₆ (NPe6): a candidate sensitizer for photodynamic therapy of tumors. *J Photochem Photobiol B Biol*. 1993; 17:135–143.
8. Roberts WG, Smith KM, McCullough JL, et al. Skin photosensitivity and photodestruction of several potential photodynamic sensitizers. *Photochem Photobiol*. 1989; 49:431–438. [PubMed: 2727082]
9. Hendrickx N, Volanti C, Moens U, et al. Up-regulation of cyclooxygenase-2 and apoptosis resistance by p38 MAPK in hypericin-mediated photodynamic therapy of human cancer cells. *J Biol Chem*. 2003; 278:52231–52239. [PubMed: 14557269]

Fig. 1.

Effects of single (5 mg/kg IP, $n = 8$) and fractionated (5 mg/kg twice, $n = 8$) doses of NPe6 without the COX-2 inhibitor on tumor weights (mg). The initial tumor weights of these 2 groups at the time of PDT were not significantly different. The left flank tumors were not exposed to light, whereas the right flank tumors received PDT. The weights of tumors not exposed to light (L flank) were not significantly different. Fractionated dosing resulted in significantly greater tumor kill than single dosing in the light-exposed right flank ($P = 0.0037$).

Fig. 2.

Effect of PDT on tumors treated with a COX-2 inhibitor ($n = 8$) only. Tumor weights at the time of PDT are not significantly different. PDT (right flank) did not have a significant effect on tumor weight.

Fig. 3.

Effects of PDT on tumors treated with NPe6 and a COX-2 inhibitor. Tumor weights at the time of PDT are not significantly different. Tumors of mice receiving NS-398 and a single dose of NPe6 ($n = 8$) showed a significant response to PDT (left flank), $P = 0.035$. Tumors exposed to NS-398 and the fractionated dose of NPe6 ($n = 8$) also showed a significant response to PDT (left flank), but the response is not significantly greater than that shown in Fig. 1.