From: PRIMARY PHOTO-PROCESSES IN BIOLOGY AND MEDICINE
Edited by R.V. Bensasson, G. Jori, E.J. Land
and T.G. Truscott
(Plenum Publishing Corporation, 1985)

HEMATOPORPHYRIN DERIVATIVE AND LIGHT PRODUCES THE VITAMIN E RADICAL

Garry R. Buettner

Laboratory of Molecular Biophysics
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

INTRODUCTION

Photodynamic therapy with hematoporphyrin derivative (HPD) is used as a treatment for malignant disease. The most active component of HPD is the di-hematoporphyrin ether (DHE).[1,2] HPD is selectively retained by tumor tissue[3,4] and exposure to red light, 630 nm, results in the photodynamic destruction of tumor tissue. Cytotoxicity has been attributed to singlet oxygen[5] and to free radicals[6].

I report here the formation of the vitamin E chromanoxyl free radical and the loss of vitamin E with the irradiation of an HPD-vitamin E solution.

MATERIALS AND METHODS

Photofrin II, a preparation of HPD with a high proportion of DHE, was purchased from Oncology Research and Development, Inc., Cheektowaga, NY, USA, and was used as received. Vitamin E (Sigma) was used as received. All solutions were prepared in absolute ethanol. Electron spin resonance (ESR) spectra were obtained with a Varian E-109 spectrometer using the aqueous sample cell. A 100 watt quartz tungsten-halogen lamp (Oriel) operating at 3200 K was employed as a white light source. The light incident on the sample was filtered through an Oriel IR blocking filter (#5205) and an Oriel long pass filter (#5130, 50% transmission cut at 530 nm). Using a Yellow Springs Instrument Model 65A radiometer and 6551 probe, the filtered light irradiance was determined to be 540 $Jm^{-2}s^{-1}$ as measured 1 cm in front of the cavity grid. Oxygen uptake was measured using the ESR linewidth technique[7] as adapted by Reszka and Chignell[8].

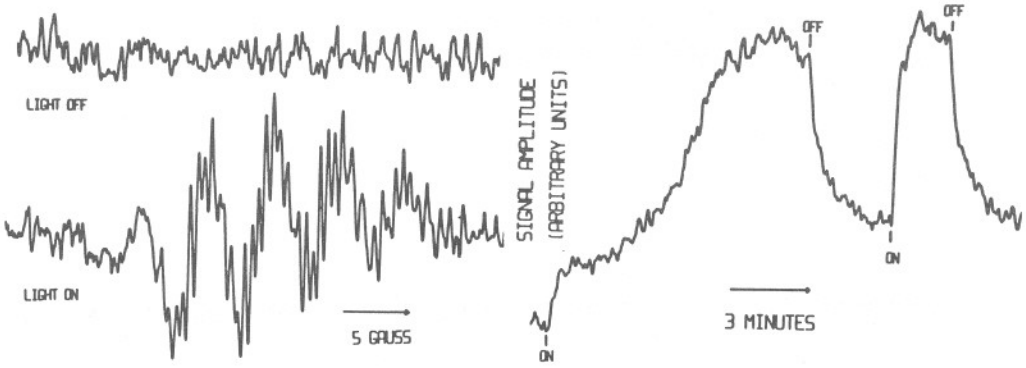

Fig. 1. Left: Chromanoxyl free radical produced during the irradiation of HPD-vitamin E solution. 0.015 M α-tocopherol with 1:50 dilution of Photofrin II in ethanol. Instrument settings: mod. amp., 0.1 G; time constant 2 s; gain, 4 x 10^5; power, 20 mW; scan 50 G/16 min. The light was turned on as the scan was started. Right: signal amplitude with time. Solution and the settings are the same as in left, except mod. amp. was 0.4 G and gain = 2.5 x 10^5. The spectrometer field was set to monitor the center multiplet of the chromanoxyl radical vs time of irradiation.

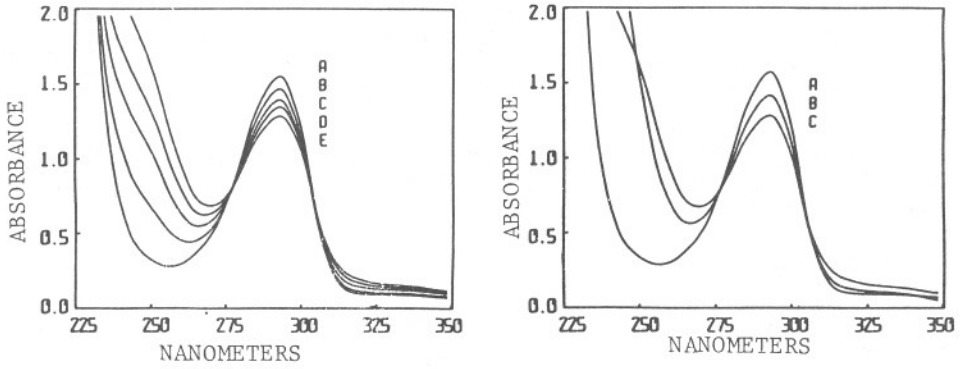

Fig. 2. Left: Irradiation of HPD-vitamin E solution for different times. HPD was present as a 1:100 dilution of Photofrin II. A. 0 min, B. 5 min, C. 10 min, D. 15 min, E. 20 min. Right: Same solution as left but B has 5 mM azide present. A. 0 min, B. 20 min, C. 20 min. The blank for the scans was a solution of HPD at the same concentration as in the irradiated samples. No loss in absorbance of the 294 nm peak was observed in the absence of HPD (not shown). No loss of HPD was noted as its absorbance at 365 nm^2 was unchanged.

RESULTS AND DISCUSSION

The irradiation of an HPD-vitamin E solution with white light

results in an ESR signal consistent with that attributed to a chromanoxyl free radical[9], see Fig. 1. Note that the first lines encountered in the scan have a much larger linewidth than the lines recorded later. This is consistent with the known oxygen broadening of ESR lines[7] and the consumption of oxygen during the irradiation of the sample. The α-tocopherol free radical appears immediately upon irradiation of the HPD-vitamin E solution, Fig. 1 right. Continued illumination results in an increase in signal amplitude, again consistent with the narrowing of the lines with the depletion of oxygen. The signal amplitude decreases when the light is off and an immediate larger increase in amplitude is observed when the light is turned on. Again, this is consistent with the depletion of oxygen from the solution. Use of the nitroxide method[7,8] showed that oxygen was consumed during irradiation.

Fig. 2 shows the loss of the characteristic absorption of vitamin E with irradiation of an HPD-vitamin E solution. The inclusion of azide ion at 5 mM inhibits the loss consistent with a role for singlet oxygen. Bubbling the solution with nitrogen prior to irradiation also inhibits the light induced loss of vitamin E (not shown).

Recently it was shown that HPD produces free radicals in the presence of ascorbate[10] and cysteine.[11] HPD-ascorbate produces hydrogen peroxide and hydroxyl free radicals with subsequent oxidation of ascorbate. HPD-cysteine produces the cysteinyl free radical and an oxygen-centered radical with the reactivity of the hydroxyl free radical. The photosensitized oxidation of tocopherols by singlet oxygen has been studied.[12,13] The observation that HPD is capable of oxidizing vitamin E in conjunction with the observed oxidation of ascorbate and cysteine suggest that photodynamic therapy may reduce the small-molecule antioxidant concentration in irradiated cells. Thus, the oxidation processes that are initiated by photodynamic therapy could easily be propagated in an environment with its antioxidant capacity compromised, leading to cell death.

ACKNOWLEDGEMENTS: GRB was a recipient of a NRSA Senior Fellowship, 3 F33 ES05285-0181. This work was supported in part by the Research Corporation and NSF equipment grants #PRM-81 08079 and TFI-80 19273.

REFERENCES

1. T.J. Dougherty, W.R. Potter and K.R. Weishaupt, The structure of the active component of hematoporphyrin derivative, in: "Porphyrin Localization and Treatment of Tumors, P.R. Doiron and C.S. Gomer, eds., A.R. Liss, Inc., New York (1984).
2. A. Poletti, S.M. Murgia, A. Pasqua, E. Reddi and G. Jori, Photophysical and Photosensitizing Properties of Photofrin II, in: "Porphyrins in Tumor Phototherapy", A. Andreoni and R. Cubeddu, eds., Plenum Press, NY (1984).

3. C.J. Gomer and T.J. Dougherty, Determination of ^{3}H and ^{14}C hematoporphyrin derivative distribution in malignant and normal tissue. Cancer Res., 30: 146 (1979).
4. H.B. Gregorie, E.O. Horger, J.L. Ward, J.F. Green, T. Richards, H.C. Robertson and T.B. Stevenson, Hematoporphyrin-derivative fluorescnece in malignant neoplasms. Ann. Surg., 167: 820 (1968).
5. K.R. Weishaupt, C.J. Gomer and T.J. Dougherty, Identification of singlet oxygen as the cytotoxic agent in photoinactivation of a murine tumor, Cancer Res., 36: 2326 (1976).
6. P.V. Hariharan, J. Courtney and S. Elecczko, Production of hydroxyl radicals in cell systems exposed to hematoporphyrin and red light, Int. J. Radiat. Biol. 37: 691 (1980).
7. J.M. Backer, V.G. Budker, S.I. Eremenko and Y.N. Molin, Detection of the kinetics of biochemical reactions with oxygen using exchange broadening in the ESR spectra of nitroxide radicals, Biochim. Biophys. Acta, 460: 152 (1977).
8. K. Reszka and C.F. Chignell, Spectroscopic studies of cutaneous photosensitizing agents-IV. The photolysis of benoxaprofen, an anti-inflammatory drug with phototoxic properties, Photochem. Photobiol., 38: 231, (1983).
9. T. Ozawa, A. Hanaki, S. Matsumoto and M. Matsuo, Electron spin resonance studies of radicals obtained by the reaction of α-tocopherol and its model compound with the superoxide ion Biochim. Biophys. Acta, 531: 72 (1978).
10. G.R. Buettner and M.J. Need, Hydrogen peroxide and hydroxyl free radical production by hematoporphyrin derivative, ascorbate and light, submitted (1984).
11. G.R. Buettner, Thiyl free radical production with hematoporphyrin-derivative, cysteine and light: a spin-trapping study, submitted (1984).
12. G.W. Grams, Oxidation of alpha-tocopherol by singlet oxygen, Tet. Lett., 50: 4823 (1971).
13. R. Yamauchi and S. Matsushita, Products formed by photosensitized oxidation of tocopherols, Agric. Biol. Chem., 43: 2157 (1979).