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Review Article

Hematoporphyrin phototherapy of cancer

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Summary

Hematoporphyrin phototherapy of cancer is a new modality for cancer diagnosis and treatment that is currently undergoing clinical trials worldwide. A variety of tumors have been studied, e.g., breast (mostly recurrent skin), lung, bladder, eye, head and neck, gynecological and brain. The most success to date has been with lung and the gynecological tract. Cell and animal studies are being conducted to elucidate the basic photobiological mechanisms involved, as well as the histopathological events associated with tumor destruction. Although major questions remain to be resolved, hematoporphyrin phototherapy is an exciting new therapeutic modality for the treatment of cancer, especially in sites where the unique features of lasers and fiber optics are advantageous.

Introduction

The selective concentration of porphyrins by malignant tissue has been known for decades, and has been thoroughly reviewed in several recent symposium volumes [4,11,20]. From these symposia, as well as the many papers published in refereed journals, a rather complex picture emerges. It has been demonstrated that (i) some porphyrins are concentrated selectively in malignant tumors in animals and man, (ii) the concentrated porphyrins can be detected by fluorescence emission, thus facilitating identification of malignancy, (iii) the concentrated porphyrins will absorb light of particular wavelengths leading to a photochemical destruction of the tumor, and (iv) in some human cases, localized tumors have been eradicated by this method, with

no regrowth detected for up to 2 years. The general scheme of porphyrin phototherapy is depicted in Fig. 1.

Despite these positive indications, the following questions remain: 1. It is not known which of the porphyrins in the complex hematoporphyrin derivative (HpD) mixture are concentrated in the malignant tissue. 2. It is not known whether the same components of HpD that fluoresce are the ones that confer photosensitivity. 3. It is not known whether singlet oxygen is really the photoproduct responsible for cell killing. 4. It is not known whether tumor destruction is a result of actual HpD phototoxicity to the proliferative cells and/or whether the tumor destruction is a result of damage produced to the tumor vasculature. 5. It is not known why some malignancies respond well and

PHOTORADIATION THERAPY OF CANCER
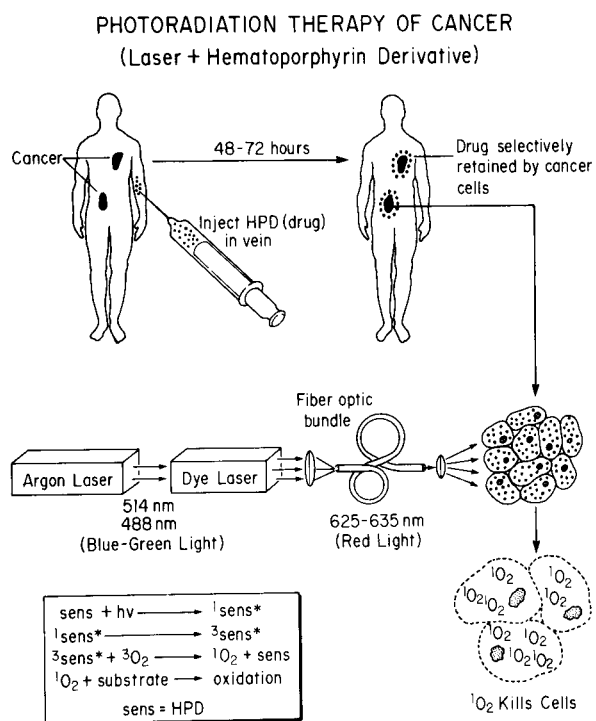### (Laser + Hematoporphyrin Derivative)

Fig. 1. Schematic representation of hematoporphyrin derivative-photoradiation therapy (HpD-PRT).

others do not. 6. It is not known which clinical situations warrant the use of hematoporphyrin phototherapy, either alone or in combination with some other modality.

Despite these apparent unanswered questions, it is clear that HpD-PRT (hematoporphyrin derivative photoradiation therapy) does work in certain situations and holds tremendous promise in cancer detection and treatment. Because of the magnitude of the cancer problem in society, as well as the often inadequate methods to treat many types and stages of cancer, any new approach that shows promise must be carefully examined. This review will deal with the current status of HpD-PRT and the progress being made to resolve some of the key unanswered questions.

Active components

It is clear from numerous studies [3,5,12, 14,15,21,24] that the HpD mixture contains numerous porphyrin components. HPLC analysis of HpD reveals a variety of peaks, some of which can be readily identified and others of which remain obscure.

With respect to the tumor localizing and photosensitizing activity of the various components, there seems to be general agreement that the greatest activity is in the broad group of components isolated in one particular region of the chromatogram [14,15,25].

Finally, attention must be given to the question of the identity of the phototoxic species. Dougherty and colleagues [13] have indicated that the active component is a deuteroporphyrinethylether (DHE). These studies have yet to be confirmed by other investigators. Interestingly, when DHE (supplied by T. J. Dougherty, Roswell Park, Buffalo, NY) or Photofrin II are run through HPLC, the same peaks are obtained as for crude HpD. The major difference is in the relative proportion of the components. In summary, it appears that considerable effort is being devoted to identification of the active component(s) of HpD. The most likely candidate for the active ingredient of the complex HpD mixture is DHE.

Fluorescence versus photosensitivity

Cells treated with HpD fluoresce [5], and tumors from animals injected with HpD fluoresce [2]. Furthermore, it is clearly documented that both bladder [2] and lung cancer [1,19] can be localized by HpD fluorescence. The real question is whether or not the fluorescent component(s) are the same as the phototoxic components. There is evidence in both directions. In vitro studies on the tumor-localizing ability of the various subcomponents suggest an inverse relationship between fluorescence activity and photosensitivity [24]. Recent studies [7,26] have demonstrated a definite correlation between in vivo tumor fluorescence activity and tumor necrosis following PRT. In the latter study, it was demonstrated that tumors with low HpD-concentrating ability (as detected by quantitative fluorescence) was insensitive to light exposure. Conversely,

a tumor with high levels of fluorescence responded well to light exposure. However, these observations do not prove conclusively that the fluorescent and phototoxic species are the same. It is still possible that the two components are different, and just follow similar patterns of uptake and concentration by the tumor tissue. In addition, Grossweiner [16] has suggested that photoactive HpD may exist in cells in both a fluorescent and nonfluorescent form.

Mechanism of tumor destruction

Studies on tissue culture cells have implicated singlet oxygen as the major photoproduct responsible for cell toxicity [33]. Recently, it has been shown rather convincingly that in an in vitro HpD liposome model system, the liposome membranes are damaged directly by singlet oxygen following exposure to light [16]. These effects are inhibited in a nitrogen-enriched, oxygen-depleted environment, and are accelerated in a ^2H_2O environment, which has been shown to prolong the lifetime of singlet oxygen. Though the evidence seems to indicate that, at the cellular level, one of the major phototoxic products is singlet oxygen, studies have not been performed to rule out other photoproducts such as free radicals.

Site of phototoxicity

The question of the site of phototoxicity is unresolved. Some studies suggest that the outer cell membrane is the target site [5,16]. However, other studies have implicated mitochondria [6,15,28], and lysosomes [30]. With respect to the mitochondria, selective damage can be demonstrated in cell cultures treated with HpD (25 μg/ml) as soon as 1 minute after laser exposure (total laser energy was 5–25 J/cm² at 625 nm). The general cell damage became progressively more severe as the time after laser exposure increased, with general nuclear pyknosis and cytoplasmic disruption evident by 12 hours following light exposure (Fig. 2A–C). This study demonstrated that the mitochondria are the
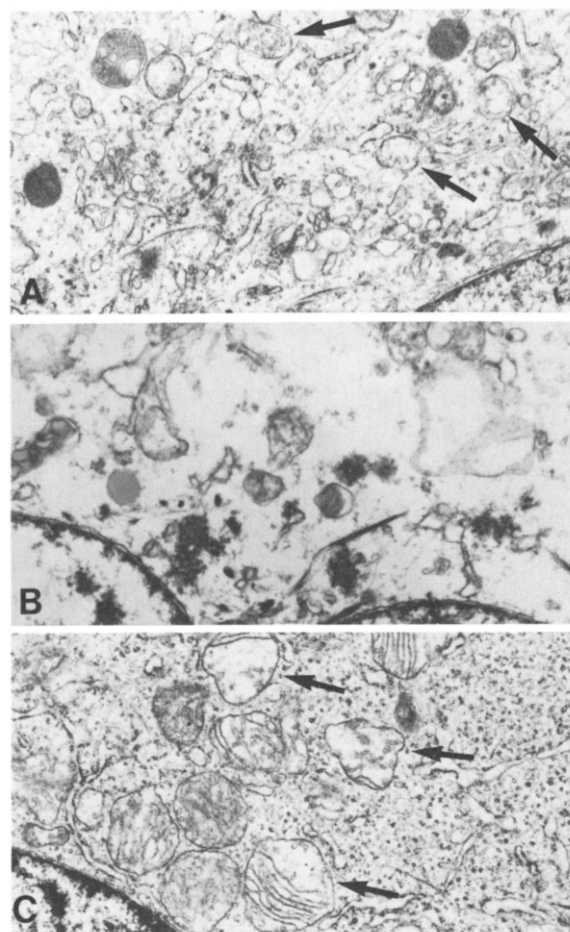

Fig. 2. A. Electron micrograph of a cell from a kidney epithelial cell culture that was exposed to HpD (25 μg/ml) for 18 hours, and then exposed to 630 nm light, 5 J/cm². The cell was fixed for electron microscopy 10 minutes after light exposure. Note selective degradation of mitochondria (arrows). Nuclear envelope and chromatin (lower right corner) are normal. Magnification, 16,000 ×. B. Similar culture and HpD-PRT exposure as in A, except the cells were fixed 12 hours after light exposure. Note the generally damaged cytoplasm, condensed pyknotic nuclei. Magnification, 14,000 ×. C. Similar cells as above, but exposed to 25 J/cm², and fixed 1 minute after light exposure. Note the selective disruption of the mitochondrial matrix and cristae. Magnification, 9600 ×.

initial cellular sites to exhibit morphological alterations. It is quite possible that there are multiple primary and secondary target sites. Until the active components are identified and their subcellular binding determined, the answer to these questions will remain unresolved.

In addition, since most of the mechanistic studies have been performed in homogeneous tissue culture systems, the question of applicability to in vivo tumors must be raised. Both the cellular heterogeneity of an in vivo tumor and the vascular supply to the tumor must be considered. Evidence exists that the primary site of damage leading to tumor destruction is actually in the tumor vasculature. This mechanism was suggested by Bugelski and colleagues [9] who demonstrated a 5-fold increase of ^{3}H-HpD in the vascular stroma in experimental animal tumors. A subsequent study came to the same conclusion [18]. In that study, cells were taken from HpD-PRT-treated animal tumors and clonal growth in vitro was determined soon after exposure to the light. No delayed or reduced growth was observed in vitro, but in vivo tumor necrosis was observed. These observations would suggest that the vasculature is destroyed, thus leading to subsequent cell death.

Another study employed a rat mammary tumor growing in an observation chamber implanted in a rat [29]. This system permitted direct microscopic visualization of the tumor vasculature. Following systemic injection of HpD, the entire tumor plus vasculature was exposed to 630 mm light. The ob-

served effect appeared to be a bleaching of the blood vessels followed by a cessation of blood flow. The authors concluded that tumor cure was a result of the destruction of microcirculation followed by tumor cell death.

However, contrary to the several studies just discussed, numerous tumor cell fluorescence studies do suggest that some HpD compounds reach the proliferative tissue. It seems possible that tumor destruction by HpD-PRT may be a result of a combination of vascular and proliferative cell destruction by HpD phototoxicity. Furthermore, recent studies by Waldow et al. [32] indicate a possible synergism between hyperthermia and HpD-PRT. This would suggest that a combination of modalities may, in fact, be the most effective way to destroy tumors.

Variability of tumor response and clinical trials

A substantial number of clinical trials of HpD-PRT have been undertaken which consistently demonstrate benefit for treated patients. A series of patients treated by us is representative of the results of these clinical trials and are summarized in Table

TABLE I

Results of the clinical use of laser hematoporphyrin derivative photoradiation therapy (HpD-PRT).

Site of cancer	No. of patients	No. of sites	Response				
			CR	PR	SD	NR	Unknown
Head and neck	39	114	28	42	3	34	7
Breast cancer	33	395	222	74	1	92	6
Lung cancer*	5	6	0	1	1	1	3

Data are from Ref. 35. Response was determined at the examination performed 4 weeks after illumination with laser light. A complete response (CR) was disappearance of all visible tumor. A partial response (PR) was resolution of more than half of the bulk of visible tumor. Stable disease (SD) was defined as a response of some sort with no tumor growth but less than a PR. NR is no response. An unknown response was either a tumor that was not evaluable because the tumor was infiltrating rather than exophytic or because the patient was not able to be examined 4 weeks following treatment. All tumors were estimated to be less than 2 cm in thickness at the time of light exposure, and had a measured width of no greater than 5 cm. The length of light exposure varied depending upon the power density (watts/cm^2) and the total light dose (joules/cm^2). Power density was in the range of 5–250 mW/cm^2 and total energy was 5–100 J/cm^2. Duration of light exposure ranged from 15 seconds to 1 hour.

* There were five patients treated; one patient was treated twice for a total of six treatments. Of the five sites treated, one of the sites was treated twice for a total of six sites.

I. One must bear in mind that a criterion of entry into our study has been the failure of conventional therapy. Hence, all patients were heavily pretreated with surgery, radiotherapy, and/or chemotherapy and possessed resistant tumor.

Initial attempts at using PRT have been phase I trials investigating feasibility and toxicity. Parameters of treatment in terms of total light dose (Joules/cm^2) and dose rate (mw/cm^2) were established. Toxicity has been minimal, with the other observed indirect effect of PRT being a sensitivity to bright artificial or natural light for periods of a month or more. This sensitivity is manifested by erythema and edema of the skin which is usually of brief duration.

It has been determined that light of wavelength 630 nm can penetrate only 1–2 cm of tissue with sufficient intensity to initiate the photochemical reaction necessary for anti-tumor therapy. This depth of penetration is further reduced when treating through intact skin containing melanin, which will absorb light at this wavelength. Early investigators did not appreciate the physical barriers of light penetration. Hence, any tumor of thickness greater than 2 cm or a tumor deeper than 2 cm from a body surface could not be illuminated with light of sufficient intensity to induce adequate therapeutic response.

In our study as well as those of others, there was a distinct number of cases where the tumors did not respond at all, or where some tumor sites in the same patient responded and other tumor sites did not (Fig. 3) [10,12,22]. The only systematic effort to examine some of the possible causes for varied tumor response was a study comparing the response of primary tumors and metastatic tumors in the head and neck [34]. It can be seen in Tables II and III that considerably more favorable responses to HpD-PRT were achieved in the primary site tumors.

Numerous factors may determine the response of any given tumor site or patient. Some of these determinants, such as general patient health and variations in metabolism, may actually affect the serum levels of HpD and ultimately the amount of HpD that reaches the tumor. For this reason, studies
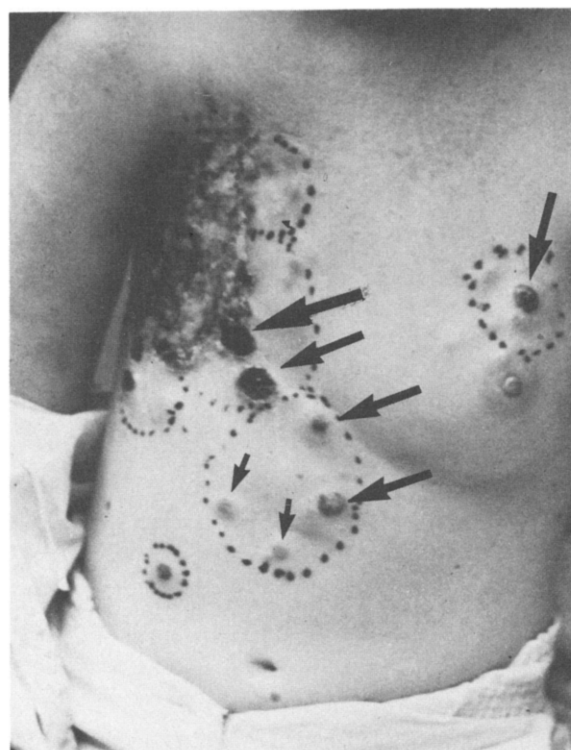

Fig. 3. Patient with extensive chest wall recurrence of breast cancer treated by HpD-PRT 24 hours earlier. Note differential response of tumor nodules, large arrows indicate necrotic response of tumor nodules, small arrows indicate tumor nodules that did not respond to the phototherapy.

should be conducted that correlate serum (and possibly urine) content of HpD with tumour content. If positive correlations are detected, then HpD-PRT can be tailored to individual patient conditions.

Another source of variability may be in the amount of vascularity associated with different types of tumors and tumor locations. The importance of the vasculature in terms of HpD uptake and excretion as well as in nourishment of the tumor has already been discussed. In fact, it is possible that much of the variable response observed in the clinical trials may be due to variations in tumor vasculature. Indeed, this could explain why different tumor sites in the same patient may respond quite differently. This could also provide a partial explanation of why different total light doses

TABLE II

Response of head and neck cancer recurrent in primary site

Tumor site	No. of patients	CR	PR	SD	NR
Tongue	9	2	6	0	1
Nasopharynx	3	1	1	0	1
Floor of mouth	2	1	1	0	0
Soft palate	2	1	1	0	0
Oropharynx	1	0	1	0	0
Buccal mucosa	1	1	0	0	0
Maxilla	1	0	1	0	0
Vocal cord	1		1		
Nevus syndrome	1		1		
Total	21	6	13	0	2

Data are from Ref. 34. Abbreviations are as in Table I.

are needed to affect tumors in the skin (10–20 J/cm^2) [10] versus tumors in the lung (50–200 J/cm^2) [1,17,22,35].

Another major source of variability is in the HpD itself. Currently no standard methods of handling the drug are followed by the different investigators (such as refrigeration, storage in the dark, utilization of material once the vial is opened). In addition, variation in the fluorescent and cytotoxic activity of different lots of the same drug may be significant. Until more standardized methods of drug preparation and handling are adopted, this source of variation in tumor response will be present. However, current efforts by the U.S. Manufacturer of HpD (Photofrin II) are aimed at standardizing the production and handling of the compounds.

In addition to drug variability, there is a large variation in the actual equipment associated with light delivery. This may be in the form of different lasers used, shifts in wavelengths emitted by the dye lasers, and actual variation in power and energy output from the laser during or between treatments.

TABLE III

Response of head and neck cancer metastatic to soft tissues of head and neck

	No. of patients	CR	PR	SD	NR
Tonsil	3	1	1	0	1
Larynx	1	0	1	0	0
Tongue	2	0	0	0	1
Parotid	1	0	0	1	0
Gingiva	2	1	0	0	0
Skin	1	0	0	0	1
Floor of mouth	1	0	0	0	1
Total	11	2	2	1	4

Data are from Ref. 34. Abbreviations are as in Table I.

The methods used by different investigators to determine power and energy densities at the tumor site also are variable. Similarly, the variation in fiber optic delivery systems may be substantial and, therefore, affect the success of the treatment. This variation may be in several areas: (i) type of fiber used (quartz, glass, diameter), (ii) preparation of fiber tips (cut, polished, etc.), (iii) geometry of fiber tips (flat cut, bulb, diffusing, angle of divergence). The way in which the fiber delivers the light to the tumor also will play a major role in tumor response: interstitial placement of the fiber versus external surface exposure of the tumor. Finally, the ultimate durability of the fiber will be important in its successful use. For example, we have used fibers that have had a light-diffusing material annealed along a certain length of its tip. The stability of this material has been quite variable, and in the case of one patient it actually deteriorated during the treatment resulting in substantial heat damage in malignant and surrounding normal tissue. However, despite all the variations that can occur with respect to the equipment, there appear to be real efforts underway to standardize all of these variables just mentioned.

With respect to the worldwide clinical application of HpD-PRT, a recent review [4] listed 14 investigators in the U.S. involved in clinical trials using HpD-PRT. It has been estimated that 1500–2000 patients have been treated worldwide [12], with nine U.S. institutions accounting for over 500 patients, and 900 patients from other countries (500 from Japan alone at 7–10 centers) listed as receiving HpD-PRT. These tumors have been of a variety of types, including breast (mostly recurrent to skin), skin (mostly basal cell carcinoma, squamous cell carcinoma and melanoma), lung, bladder [12], eye [8], head and neck [34], gynecological [27], and brain [23].

Though most of the human treatments have been on advanced stage malignancy, results are now being obtained on earlier malignancies such as carcinoma in situ of the bladder [2,31], lung [17], and gynecological tract [27]. In the case of the lung and gynecological tract, a few patients treated only with HpD-PRT are approaching 2 years without evidence of recurrence of the disease.

In summary, HpD-PRT has been demonstrated to be a viable therapeutic modality in the management of cancer. It is a modality which has only recently completed its phase I trial and is beginning its phase II trials evaluating efficacy in selected sites of human malignancy. It is apparent that parameters of treatment can and should be standardized. It is also apparent that tumors will respond to HpD-PRT even after failure of multiple modality therapy. Even though the future role of HpD-PRT in the diagnosis and treatment of malignancy is unclear at the present time, this new modality could have a significant impact.

Acknowledgements

References

1 Balchum, O. J., Doiron, D. R. and Huth, G. C. Photoradiation therapy of endobronchial lung cancers employing the photodynamic action of hematoporphyrin derivative. Lasers Surg. Med. 4: 13–30, 1984.

2 Benson, R. C., Farrow, G. M., Kinsey, J. H., Cortese, D. A., Zincke, H. and Utz, D. C. Detection and localization of in situ carcinoma of the bladder with hematoporphyrin derivative. Mayo Clin. Proc. 57: 548–555, 1982.

3 Berenbaum, M., Bonnett, R. and Scourides, P. A. In vivo biological activity of the components of hematoporphyrin derivative. Brit. J. Cancer 45: 571–581, 1982.

4 Berns, M. (Ed.). Hematoporphyrin Derivative Photoradiation Therapy, Lasers Surg. & Med. Vol. 4, New York: Alan R. Liss, 1984.

5 Berns, M. W., Hammer-Wilson, M., Walter, R. J., Wright, W., Chow, M.-H., Nahabedian, M. and Wile, A. Uptake and localization of HpD and "active fraction" in tissue culture and in serially biopsied human tumors. Prog. Clin. Biol. Res. 170: 501–520, 1984.

6 Berns, M. W., Dahlman, A., Johnson, F. M., Burns, R. G., Sperling, D., Guiltinan, M., Siemens, A., Walter, R. J., Wright, W. and Hammer-Wilson, M. In vitro cellular effects of hematoporphyrin derivative (HpD). Cancer Res. 42: 2325–2329, 1982.

7 Boggan, J. E., Walter, R. J., Edwards, M. S. B., Borich, J.

240

K., Davis, R. L., Koonce, M. and Berns, M. W. Distribution of HpD in the rat 9L gliosarcoma brain tumor as analyzed by digital video fluorescence microscopy. J. Neurosurg. 61: 1113–1119, 1984.

8 Bruce, Robert, A. Evaluation of hematoporphyrin photoradiation therapy to treat choroidal melanomas. Lasers Surg. Med. 4: 59–64, 1984.

9 Bugelski, P. J., Porter, C. W. and Dougherty, T. J. Autoradiographic distribution of hematoporphyrin derivative in normal and tumor tissue of the mouse. Cancer Res. 41: 4606–4612, 1981.

10 Dahlman, A., Wile, A. G., Burns, R. G., Mason, G. R., Johnson, F. and Berns, M. W. Laser photoradiation therapy of cancer. Cancer Res. 43: 430–434, 1982.

11 Doiron D. and Gomer, C. J. (Eds.) Prog. Clin. Biol. Res., Vol. 170, New York: Alan R. Liss, 1984.

12 Dougherty, T. J. An overview of the status of photoradiation therapy. Prog. Clin. Biol. Res. 170: 75–87, 1984.

13 Dougherty, T. J., Boyle, D. G., Weishaupt, K. R., Henderson, B. A., Potter, W. R., Bellnier, D. A. and Wityk, K. E. Photoradiation Therapy — Clinical and Drug Advances. Adv. Exp. Med. Biol. 160: 3–13, 1983.

14 Evensen, J., Sommer, S., Moan, J. and Christensen, T. Tumor localizing and photosensitizing properties of main components of hematoporphyrin derivative. Cancer Res. 44: 482–486, 1984.

15 Gibson, S. L., Leakey, P. B., Crute, J. J. and Hilf, R. Photosensitization of mitochondrial cytochrome c oxidase by hematoporphyrin derivative (HpD) in vitro and in vivo. Prog. Clin. Biol. Res. 170: 323–334, 1984.

16 Grossweiner, L. I. Membrane photosensitization by hematoporphyrin and hematoporphyrin derivative. Prog. Clin. Biol. Res. 170: 391–404, 1984.

17 Hayata, Y., Kato, H., Konaka, C., Ono, J., Amemiya, R., Kinoshita, K., Sakai, H. and Yamada, R. Hematoporphyrin derivative and photoradiation therapy in early stage lung cancer. Lasers Surg. Med. 4: 39–48, 1984.

18 Henderson, B. W., Dougherty, T. J. and Malone, P. B. Studies on the mechanism of tumor destruction by photoradiation therapy. Prog. Clin. Biol. Res. 170: 601–612, 1984.

19 Kato, H., Aizawa, K., Ono, J., Konaka, C., Kawate, N., Yoneyama, K., Kinoshita, K., Nishimiya, K., Sakai, H., Noguchi, M., Tomono, T., Kawasaki, S., Tokuda, Y. and Hayata, Y. Clinical measurement of tumor fluorescence using a new diagnostic system with hematoporphyrin derivative, laser photoradiation, and a spectroscope. Lasers Surg. Med. 4: 49–58, 1984.

20 Kessel, D. and T. J. Dougherty (Eds.). Porphyrin Photosensitization, New York: Plenum Publishers, 1983.

21 Kessel, D. Chemical and biochemical determinants of porphyrin localization. Prog. Clin. Biol. Res. 170: 405–418, 1984.

22 Li, J.-H, Chen, Y.-P., Zhao, S.-D., Zhang, L.-T. and Song,

S.-Z. Application of hematoporphyrin derivative and laser-induced photodynamical reaction in the treatment of lung cancer: A preliminary report on 21 cases. Lasers Surg. Med. 4: 31–38, 1984.

23 McCulloch, G. A. J., Forbes, I. J., See, K. L., Cowled, P. A., Jacka, F. J. and Ward, A. D. Phototherapy in malignant brain tumors. Prog. Clin. Biol. Res. 170: 709–717, 1984.

24 Moan, J., Christensen, T. and Sommer, S. The main photosensitizing components of hematoporphyrin derivative. Cancer Lett. 55: 161–166, 1982.

25 Moan, J., Christensen, T. and Jacobsen, P. B. Porphyrin-sensitized photoinactivation of cells in vitro. Prog. Clin. Biol. Res. 170: 419–442, 1984.

26 Nelson, J. S., Wright, W. H. and Berns, M. W. Histopathological comparison of the effects of hematoporphyrin derivative on two different murine tumors using computer enhanced digital video fluorescence microscopy. Cancer Res. 45: 5781–5786, 1985.

27 Rettenmaier, M. A., Berman, M. L., DiSaia, P. J., Burns, R. and Berns, M. W. Photoradiation therapy of gynecologic malignancies. Gynecol. Oncol. 17: 200–206, 1984.

28 Salet, C. and Moreno, G. Photodynamic effects of hematoporphyrin derivative (HpD) on respiration and calcium uptake in isolated mitochondria. Int. J. Rad. Biol. 39: 227–230, 1981.

29 Star, W. M., Marijnissen, J. P. A., Van den Berg-Blok, A. E. and Reinhold, H. S. Destructive effect of photoradiation on the microcirculation of a rat mammary tumor growing in "sandwich" observation chambers. Prog. Clin. Biol. Res. 170: 637–645, 1984.

30 Torinuki, W., Miiura, T. and Seiji, M. Lysosome destruction and lipopreoxide formation due to active oxygen generated from haematoporphyrin and uv irradiation. Brit. J. Dermatol. 102: 17–27, 1980.

31 Tsuchiya, A., Obara, N., Makoto, M., Ohi, T., Kato, H. and Hayata, Y. Hematoporphyrin derivative and laser photoradiation in the diagnosis and treatment of bladder cancer. J. Urol. 130: 79–82, 1982.

32 Waldow, S. M., Henderson, B. W. and Dougherty, T. J. Enhanced tumor control following sequential treatments of photodynamic therapy (PDT) and localized microwave hyperthermia in vivo. Lasers Surg. Med. 4: 79–86, 1984.

33 Weishaupt, K. R., Gomer, C. J. and Dougherty, T. J. Identification of singlet oxygen as the cytotoxic agent in photoinactivation of a murine tumor. Cancer Res. 36: 2326–2329, 1976.

34 Wile, A., Novotny, J., Mason, G. R., Passy, V. and Berns, M. W. Photoradiation therapy of head and neck cancer. Am. J. Clin. Oncol. 6: 39–43, 1984.

35 Wile, A. G., Coffey, J., Nahabedian, M. Y., Baghdassarian, R., Mason, G. R. and Berns, M. W. Laser photoradiation therapy of cancer: An update of the experience at the University of California, Irvine. Lasers Surg. Med. 4: 5–12, 1984.