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Determination of [^{3}H]- and [^{14}C]Hematoporphyrin Derivative Distribution in Malignant and Normal Tissue[1]

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ABSTRACT

The synthesis and tissue-localizing ability of [^{14}C]- and [^{3}H]hematoporphyrin derivative (HPD) in mice have been described. Tissue levels and distributions were the same for both radioactive compounds, indicating that *in vivo* tritium exchange did not occur with [^{3}H]HPD. The amount of [^{14}C]HPD or [^{3}H]HPD which localized in the transplanted tumor tissue of mice at various times following i.p. injection (10 mg/kg) was higher than in skin or muscle tissue but was less than in liver, kidney, or spleen tissue. These results tend to disprove the generalization that HPD accumulates in malignant tissue to a higher degree than in all normal tissue. It is also reported that gross visualization of porphyrin fluorescence cannot be correlated with actual tissue concentrations of the dye.

INTRODUCTION

Studies utilizing HPD[4] have been of increasing interest primarily because of the potential usefulness of the dye in both the diagnosis and treatment of malignant disease. The property of selective localization of HPD in malignant tissue can be exploited for the fluorescent delineation of solid tumors, while the photodynamic action of the compound can be utilized to destroy malignant tissue selectively while leaving surrounding normal tissue virtually undamaged.

Initial studies using a crude hematoporphyrin as a tumor-localizing drug were performed in 1942, when it was observed that injected hematoporphyrin tended to accumulate specifically in neoplastic tissue in rats (1). In 1961, HPD was prepared by treating the crude material with a mixture of acetic and sulfuric acid (11). It was observed that HPD exhibited improved and more reproducible tumor-localizing abilities. With 15 patients having malignant tumors of the esophagus or tracheobronchial tree, positive fluorescence was demonstrated in every case approximately 3 hr following infusion of HPD (2 mg/kg) (12). In contrast to the red fluorescence of the tumor, the normal tissue exhibited a grayish white appearance.

In an extensive clinical study utilizing HPD as a diagnostic tool, 226 patients were examined for HPD fluorescence following i.v. administration of 1000 mg of HPD (7). In 76% of the 173 lesions diagnosed positively as malignant by histological examination, the tumors were found to be fluorescent when illuminated 2 hr following the administration of the dye. The surrounding normal tissue was found to be free of fluorescence. In a separate study, 40 patients having lesions of the oral cavity, pharynx, hypopharynx, and larynx were examined for fluorescence after receiving HPD (2 mg/kg) (10). Positive tumor fluorescence was found in all 29 patients confirmed to have a malignant disease, whereas the 11 other patients in which no fluorescence occurred proved to have benign lesions.

Diagnostic studies to attempt to locate microscopic lung tumors by using HPD fluorescence have been described (14). Optical improvements in the fiberscopes necessary for detection of small amounts of fluorescence are also being carried out (13).

The therapeutic potential of HPD utilizing its photodynamic action along with its tumor specificity was first demonstrated in 1972 when glioma tumors transplanted into rats responded destructively to the combined effects of HPD and visible light (2). In 1975, it was reported that low doses of HPD followed 24 hr later by exposure to red light could be used to produce cures of spontaneous and transplanted mammary tumors in mice and rats (4). It was subsequently reported that the cytotoxicity induced by HPD results from the intracellular formation of singlet oxygen (a short-lived, highly reactive state of the oxygen molecule) when cells containing the dye were exposed to visible light (16).

An extensive clinical trial using HPD photoradiation therapy is in progress (3, 5). Administration of HPD i.v. followed 3 to 4 days later by local exposure to red light resulted in complete or partial tumor response in 21 out of 25 patients having cutaneous or s.c. malignant lesions. More than 100 individual tumors of at least 10 different histological types have been treated. No type of lesion was found to be characteristically unresponsive. Very high therapeutic ratios between tumor response and skin response were observed.

Despite these laboratory and clinical observations attesting to the potential usefulness of HPD because of its ability to concentrate preferentially in malignant tissue, there have been no in-depth quantitative data regarding this phenomenon. The 2 attempts to quantitate *in vivo* hematoporphyrin and HPD concentrations centered around only 2 tissues, tumor and muscle or tumor and liver (17, 4). Both studies involved tedious tissue extraction procedures followed by porphyrin determinations made by fluorometric and spectrophotometric techniques. Because of the difficulties involved in obtaining complete extraction of hematoporphyrin or HPD from tissue, combined with the possible fluorescent quenching by tissue components, the accuracy and reproducibility of these results are questionable.

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[4] The abbreviation used is: HPD, hematoporphyrin derivative.

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This paper describes the preparation of [^{14}C]- and [^{3}H]HPD and subsequent tests performed to analyze for their comparability to the standard nonradioactive HPD. Data are presented regarding the quantitative distribution of these radioactive compounds in 8 benign and malignant tissues at various time periods following administration in 2 mouse strains.

MATERIALS AND METHODS

Animal and Tumor Systems

Eight- to 12-week-old female DBA/2 Ha-DD mice into which a spontaneous mammary carcinoma was transplanted (8) or C3Hf/Sed-BH mice into which methylcholanthrene mammary carcinoma was transplanted (15) were used in all *in vivo* tissue distribution and photoradiation experiments. Transplanted tumors were initiated by trochar injection of approximately 1-cu mm pieces of fresh tumor into the axillary region of the mice.

Preparation of HPD

HPD was prepared by a modification of the method of Lipson (11) by dissolving one part crude hematoporphyrin hydrochloride (Roussell Corp., New York, N. Y.) in acetic acid:sulfuric acid (19:1, by volume). The solution was stirred for 1 hr at room temperature, filtered, and then precipitated using 3% sodium acetate. The resulting HPD was filtered, washed with distilled water, and dried. An injectable solution of this material was prepared by dissolving 1 g of the solid HPD in 50 ml of 0.1 N sodium hydroxide. After 1 hr of stirring, the solution was neutralized to pH 7.1 with 0.1 N hydrochloric acid and then adjusted to a total volume of 200 ml with 0.9% NaCl solution. The concentration of the solution of HPD was 5 mg/ml.

Preparation of [^{14}C]HPD

Hematoporphyrin is manufactured commercially from the degradation of hemoglobin. Inasmuch as glycine is one of the 2 precursors of 5-aminolevulinic acid at the initiation of heme biosynthesis, by utilizing [2-^{14}C]glycine it is possible for the radioactive carbon atom to be incorporated into any of 8 positions in the basic ring structure of the heme molecule and, therefore, of hematoporphyrin (Chart 1).

One female New Zealand White rabbit (3 kg) was placed in a hypoxic chamber (10% oxygen) to stimulate erythropoiesis. Following 1 week in the chamber, the animal was started on a multiinjection schedule of [2-^{14}C]glycine (55 mCi/mmol; Amersham, Arlington Heights, Ill.). One i.v. injection, consisting of 1 mCi of the material, was administered each day for 4 days. Five days after the last injection, the rabbit was bled, and 130 ml of whole blood were obtained.

[^{14}C]Hemin was then extracted from the whole blood using the procedure of Labbe and Nishida (9). The blood was added to 1200 ml of extraction solution (300 ml of 2% $SrCl_2$ in acetic acid and 900 ml acetone). This mixture was stirred for 30 min, heated to a boil, and then allowed to cool to room temperature. The mixture was filtered with suction through a sintered glass filter. The filtrate was transferred

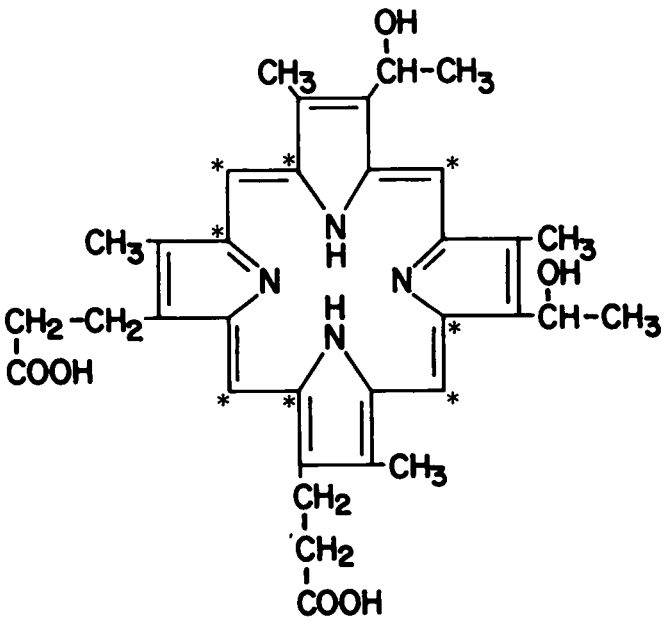

Chart 1. Structure of [^{14}C]hematoporphyrin. *, possible positions of ^{14}C incorporation.

to a beaker and heated to 100°. Crystallization of the hemin was started and became complete when the solution was allowed to cool to room temperature. The hemin was then collected after centrifugation and washing (twice with 50% acetic acid and water, and once each with ethanol and ethyl ether). The total yield of hemin was 510 mg.

The preparation of [^{14}C]hematoporphyrin (crude material) from [^{14}C]hemin was performed using the method of Granick and Bogorad (6). The hemin was shaken for 24 hr in 100 ml of hydrobromic acid (30 to 32% in acetic acid). The mixture was then poured into 700 ml distilled water and, after allowing a few min for hydrolysis of the HBr adduct, it was then neutralized to pH 6.0 with concentrated sodium hydroxide solution. The crude hematoporphyrin precipitate was filtered and washed several times with distilled water.

The crude [^{14}C]hematoporphyrin was then converted to [^{14}C]HPD according to the method described above. An injectable solution of the [^{14}C]HPD was prepared by adding 3.0 ml of 0.1 N sodium hydroxide to 20 mg of [^{14}C]HPD. After allowing the solution to stand for 1 hr, it was neutralized to pH 7.1 with 2.4 ml 0.1 N hydrochloric acid and then diluted to 10 ml with 0.9% NaCl solution. Additional sodium chloride was then added to make the solution isotonic. The final solution of [^{14}C]HPD was 2 mg/ml and had a specific activity of 27.2 μCi/mmol (100 dpm/μg).

Preparation of [^{3}H]HPD

[^{3}H]HPD was obtained by subjecting a 75-g sample of HPD to tritium labeling via the technique of catalytic exchange labeling (performed by New England Nuclear, Boston, Mass.). The HPD was dissolved in 0.8 ml of dry dimethylformamide. To this were added 25 mg of 5% Rh/Al_2O_3 and 10 Ci of tritiated water. The reaction mixture was stirred overnight at 70°. Labile tritium was removed in a vacuum using methanol as solvent. After filtration from the catalyst, the compound was again taken to dryness in a

vacuum and then taken up in10 ml of chloroform. Following catalytic exchange labeling, the purity of the [^{3}H]HPD was assessed by thin-layer chromatography in a methanol: benzene (40:60, v/v) solvent system. In addition to the [^{3}H]HPD, thin-layer chromatography demonstrated both mono and dimethyl esters of [^{3}H]HPD which accounted for approximately 5 to 10% of the derivative. Upon dissolving the [^{3}H]HPD in 0.1 N sodium hydroxide and neutralizing to pH 7.2 with 0.1 N hydrochloric acid, the methyl esters were hydrolyzed, and the resulting chromatographic pattern was identical to that of cold HPD. Radioactivity was assayed to be present only in the 3 chromatographic bands representative of HPD.

A 5-mCi ampul of [^{3}H]HPD (4.3 mg) in chloroform was then evaporated to dryness. Cold HPD was added to bring the total concentration of the dye to 20 mg. Two ml of 0.1 N sodium hydroxide were added, and the resulting solution was allowed to stand at room temperature for 1 hr. The solution was neutralized to pH 7.0 with 0.1 N hydrochloric acid and was then diluted to a final volume of 10 ml with 0.9% NaCl solution. The specific activity of this [^{3}H]HPD was 90 mCi/mmol or 3.3×10^5 dpm/μg. This solution was used in all [^{3}H]HPD tissue distribution studies.

Tests for Comparability of HPD, [^{14}C]HPD, and [^{3}H]HPD

a) Absorption Spectra. Equal weights of HPD, [^{14}C]HPD, and [^{3}H]HPD were dissolved in equal volumes of methanol, and absorption spectra were determined using a Beckman Model 25 spectrophotometer. The resulting absorption peaks from 360 to 700 nm were compared for each derivative in terms of characteristic wavelength and extinction coefficient.

b) Thin-Layer Chromatography. Acid precipitates (pH 3.5) of solutions of HPD, [^{14}C]HPD, and [^{3}H]HPD were dissolved in acetone and then run on Polygram Sil N-HR precoated plastic sheets in a solvent mixture of 40% methanol and 60% benzene. The resulting chromatographic patterns for 3 derivatives were compared. Thin-layer chromatography of HPD was carried out in several solvent systems. Adequate separation of the various components of HPD, however, was achieved only with the methanol:benzene solvent systems.

c) Biological Activity. The *in vivo* tumor response to photoradiation treatment as reported previously (4) was studied in tumorous DBA/2 Ha-DD mice using either HPD, [^{14}C]HPD, or [^{3}H]HPD. When tumors were 5 to 7 mm in diameter, the animals received an i.p. injection (10 mg/kg) of one of the compounds, and 24 hr later their tumors were exposed for 1 hr to red light (600 to 700 nm) from a 1000-watt Xenon arc lamp at an intensity of 100 milliwatts/sq cm. Each animal received 3 such treatments (dye followed by light) in 1 week. During and after the treatments, the animals were observed for tumor response. Results obtained for each of the derivatives were then compared.

d) *In Vivo* Tissue Distribution. The tissue distributions obtained using [^{14}C]HPD were compared directly to tissue distributions obtained using [^{3}H]HPD. Equal doses of either of the radioactive derivatives were administered to tumorous DBA/2 Ha-DD mice.

[^{14}C]HPD and [^{3}H]HPD Tissue Distribution Measurements

When the tumors in the mice reached appropriate sizes (5 to 7 mm in diameter), i.p. injections (10-mg/kg doses of either [^{14}C]HPD or [^{3}H]HPD) were administered. Animals were then anesthetized with ether at time periods from 1 to 72 hr postinjection. Whole blood (0.2 ml) and tissue samples (50 to 300 mg) of tumor, liver, kidney, spleen, lung, skin, and muscle were collected. One sample of each tissue was obtained for each mouse. The wet weight of each tissue was recorded and, following tissue combustion using a Packard Tri-Carb Model 306 sample oxidizer, the total ^{14}C or ^{3}H content of the sample was determined by standard liquid scintillation counting using a Beckman Model LS-3100 liquid scintillation counter. Tissue samples from mice into which HPD was injected were also oxidized and counted; these served as background measurements. The resulting cpm for each sample were converted to dpm and then converted to weights of radioactive HPD by oxidizing known concentrations of [^{14}C]HPD or [^{3}H]HPD for each experiment. Although it would have been desirable, we were unable to extract the labeled material from tissues to determine its exact composition.

RESULTS

Comparability of HPD, [^{14}C]HPD, and [^{3}H]HPD. No variations could be detected among the 3 HPD's from the tests which were performed to determine the degree of their similarity. Identical absorption spectra were obtained for the 3 derivatives. Molar extinction coefficients obtained in methanol at 395, 497, 529, 567, and 621 nm were 100,467, 9,807, 5,980, 4,186, and 2,631 liters/mol/cm, respectively. Thin-layer chromatographic patterns for the 3 compounds exhibited the same 3 bands (33, 50, and 12% in increasing R_F) which are characteristic of the acid precipitate of HPD (T. J. Dougherty, unpublished results). Tumor responses in the mice into which were injected either [^{14}C]HPD, [^{3}H]HPD, or HPD and which were followed 24 hr later by red light treatment were all qualitatively the same. All tumors responded strongly to the treatment, resulting in marked regression of the tumor and the formation of a scab over the exposed tumor area. The tissue distribution measurements comparing [^{14}C]HPD and [^{3}H]HPD are seen in Chart 2. Average tissue levels of [^{14}C]HPD and [^{3}H]HPD obtained at 24, 48, and 72 hr following drug administration are plotted for tumorous DBA/2 Ha-DD mice. There was excellent agreement between the [^{14}C]HPD tissue levels and the [^{3}H]HPD tissue levels at each time period. Error bars were not included on the chart for increased clarity but all S.D.'s of the mean for corresponding [^{14}C]HPD and [^{3}H]HPD levels overlapped, indicating no statistical difference.

Tissue Distribution Studies. The results of the blood levels and tissue distribution studies using [^{14}C]HPD and [^{3}H]HPD are shown in Tables 1 and 2. Tissue distribution levels for [^{14}C]HPD were the same for both DBA/2 Ha-DD and C3Hf/Sed-BH mice. Of the 7 tissues which were assayed for porphyrin content, the liver, kidney, and spleen (for each time period) contained higher quantities of the radioactive derivatives than did the tumor. In addition, it

was not until 24 hr after injection that porphyrin levels in the lung fell below that of the tumor, and this lasted only until 72 hr postinjection, at which time tumor and lung levels of the dye became equal. The amount of [^{14}C]HPD or [^{3}H]HPD localized in the tumor at each time period following injection was always higher than in corresponding skin or muscle tissue. In contrast to these quantitative levels were the observations made of porphyrin fluorescence in tissue. In mice sacrificed 24 hr following injection of either [^{14}C]HPD or [^{3}H]HPD and then dissected and exposed to blue light (390 to 450 nm), there was bright red fluorescence in and surrounding the tumor mass. No fluorescence was observed in any other tissue including liver or spleen.

From Table 1, it can be determined that [^{14}C]HPD values reach a maximum in the blood at 2 to 3 hr following an i.p. injection and that the half-life of the dye in the blood is approximately 3 hr. Maximum porphyrin levels in all tissues analyzed (with either mouse strain or radioactive derivative) were usually obtained by 3 or 4 hr. This was followed for the next 44 or 45 hr by a plateau in accumulation which exhibited only slight variation from the 4-hr values.

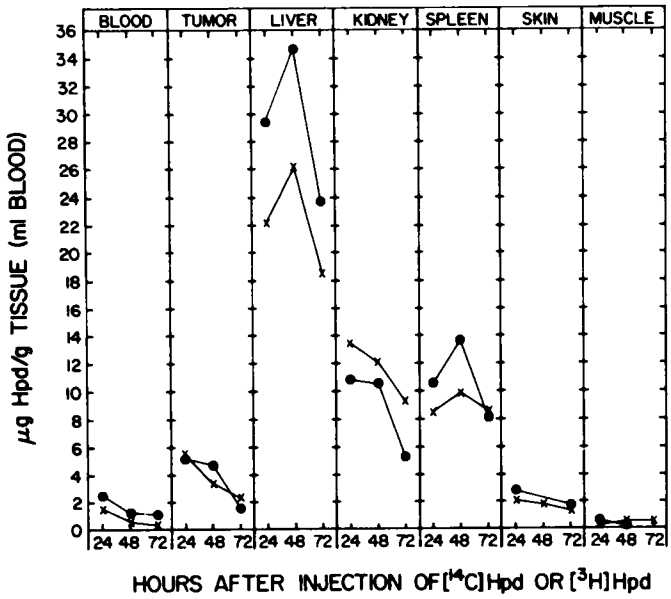

Chart 2. Tissue distribution of [^{14}C]HPD and [^{3}H]HPD in tumorous DBA/2 Ha-DD mice at 24, 48, and 72 hr following i.p. injection (10 mg/kg). ●, [^{14}C]HPD; ×, [^{3}H]HPD.

DISCUSSION

The assumption that [^{14}C]HPD was a good mimic of HPD in terms of *in vivo* tissue distribution was based on the facts that the ^{14}C atoms were incorporated only in the stable ring structure of the porphyrin molecule and that no biological or chemical differences could be detected between [^{14}C]HPD and HPD. It was necessary to obtain tissue distribution levels using [^{14}C]HPD before utilizing the [^{3}H]HPD because, if only [^{3}H]HPD was used to determine tissue distributions, the results would be less reliable from our not knowing whether the radioactive label remained exclusively attached to the porphyrin molecule. The excellent agreement between [^{14}C]HPD and [^{3}H]HPD tissue levels indicates that very little if any tritium exchange had occurred.

These results are in contrast to those reported for studies examining tissue distribution following administration of [^{14}C]- and [^{3}H]tetraphenylporphinesulfonate (18). In those experiments, [^{3}H]tetraphenylporphinesulfonate exhibited entirely different tissue distribution patterns from those obtained for the ^{14}C material. The variations in distribution were explained in terms of tritium exchange. The lack of tritium exchange observed with [^{3}H]HPD may in part be due to the different hydrogen atoms in HPD (aliphatic) compared to those in tetraphenylporphinesulfonate (aromatic).

The high specific activity which is obtained with [^{3}H]HPD will allow us in the future accurately to examine tissue distribution patterns at much lower doses or, possibly more importantly, with much smaller samples of tissue. This may prove especially useful in comparing HPD distributions in various sections of tumors such as well-oxygenated viable tissue and necrotic tissue. The high specific activity of this compound is being utilized presently to perform autoradiographic and cellular fractionation experiments aimed at studying subcellular localization in both malignant and normal tissue.

Table 1

Tissue and blood distribution of [^{14}C]HPD[a] in C3Hf/Sed BH mice at various times following injection

Time after injection (hr)	μg [^{14}C]HPD/g tissue							
	Blood	Tumor	Liver	Kidney	Spleen	Lung	Skin	Muscle
1	10.31 ± 4.50[b] (2)[c]	2.67 (1)	13.70 ± 3.62 (2)	6.19 ± 0.04 (2)	6.05 ± 1.68 (2)	7.13 ± 2.39 (2)	1.40 ± 0.52 (2)	1.92 ± 0.24 (2)
2	22.01 (1)		23.03 (1)	10.84 (1)	9.98 (1)	8.70 (1)	2.80 (1)	2.70 (1)
3	21.38 ± 1.64 (2)	3.31 (1)	32.86 ± 1.58 (2)	12.67 ± 1.00 (2)	11.88 ± 0.60 (2)	9.82 ± 1.77 (2)	3.20 ± 1.30 (2)	0.98 ± 0.82 (2)
4	14.89 (1)		24.25 (1)	10.64 (1)	11.60 (1)	5.06 (1)	1.99 (1)	1.99 (1)
6	10.57 (1)		33.89 (1)	13.01 (1)	11.63 (1)	5.34 (1)	3.03 (1)	0.36 (1)
24	2.76 ± 1.59 (16)	3.40 ± 0.89 (10)	29.07 ± 4.93 (15)	8.40 ± 1.34 (16)	9.96 ± 2.95 (17)	2.03 ± 2.01 (4)	1.90 ± 1.34 (6)	1.90 ± 1.95 (18)
48	0.91 ± 0.44 (5)	4.10 ± 1.34 (5)	27.18 ± 2.67 (4)	7.16 ± 1.17 (5)	9.69 ± 4.87 (5)	2.57 (1)	2.64 ± 1.13 (3)	0.00 (5)
72	0.96 ± 0.37 (2)	2.39 ± 0.47 (2)	23.51 ± 4.12 (4)	6.34 ± 2.08 (4)	8.70 ± 3.21 (4)	1.53 ± 0.55 (2)	1.88 ± 0.26 (4)	0.00 (2)

[a] Each mouse received [^{14}C]HPD (10 mg/kg i.p.).
[b] Mean ± S.D.
[c] Numbers in parentheses, number of samples analyzed (1 sample/mouse).

Table 2

Tissue and blood distribution of [³H]HPD[a] in DBA/2 Ha DD mice at various times following injection

Time after injection (hr)	μg [³H]HPD/g tissue							
	Blood	Tumor	Liver	Kidney	Spleen	Lung	Skin	Muscle
1	6.17 (1)[b]	2.63 ± 0.55[c] (2)	13.97 ± 1.03 (2)	12.08 ± 1.04 (2)	10.66 ± 0.40 (2)	4.99 ± 1.62 (2)	2.53 ± 0.46 (2)	1.63 ± 0.35 (2)
4	9.49 ± 0.70 (3)	5.35 ± 1.28 (3)	22.17 ± 3.26 (3)	17.55 ± 0.21 (3)	9.26 ± 1.23 (3)	5.84 ± 1.02 (3)	3.20 ± 1.22 (2)	1.56 ± 0.19 (3)
17	2.15 ± 0.68 (5)	5.24 ± 1.11 (4)	22.48 ± 1.98 (5)	19.13 ± 2.23 (5)	8.79 ± 1.07 (5)			0.38 ± 0.57 (5)
24	1.48 ± 0.19 (7)	5.23 ± 0.91 (7)	22.14 ± 2.68 (7)	13.53 ± 2.25 (7)	8.53 ± 0.98 (7)	2.74 ± 0.77 (7)	2.06 ± 0.03 (2)	0.60 ± 0.45 (7)
48	0.64 ± 0.10 (3)	3.36 ± 0.72 (3)	26.17 ± 2.38 (3)	12.06 ± 0.88 (3)	9.90 ± 0.10 (3)	2.52 ± 0.27 (3)	1.96 ± 0.46 (3)	0.87 ± 0.20 (3)
72	0.38 ± 0.07 (2)	2.20 ± 0.01 (2)	18.56 ± 2.60 (2)	9.25 ± 3.56 (2)	8.36 ± 1.59 (2)	2.17 ± 0.21 (2)	1.42 ± 0.66 (2)	0.93 ± 0.31 (2)

[a] Each mouse received [³H]HPD (10 mg/kg i.p.).
[b] Numbers in parentheses, number of samples analyzed (1 sample/mouse).
[c] Mean ± S.D.

From the results obtained using radioactive HPD, it has been demonstrated that, whereas mouse tumors tended to accumulate HPD and exhibit fluorescence, the specificity of HPD for malignant tissue appears not to be as great as indicated previously from studies based on relative tissue fluorescence. Fluorescence suffers from the fact that its apparent degree of brightness depends upon the depth of penetration of the activating light (generally blue) into the tissue as well as the transmission of the fluorescent wavelengths (red). These properties vary considerably depending upon the degree of pigmentation of the tissues. Thus, although the lightly pigmented tumors studied here show a moderate degree of fluorescence, the darkly pigmented liver, spleen, and kidney exhibited essentially none, in spite of the fact that they contained several times as much HPD. In addition, fluorescence quenching to various degrees may be occurring in one or more of these tissues.

In spite of the high concentrations of HPD in certain tissues, the higher selectivity of the dye compared to skin is sufficient to allow a high therapeutic ratio to be obtained when these tumors (and a margin of surrounding skin) are exposed to red light 24 hr following administration of HPD. As has been shown previously, 50% of such tumors can be cured by this photoradiation technique with essentially no damage to normal skin within the light field (4). We have also shown that the transplantable Brown-Pearce tumor grown in the intestine of Belted Dutch rabbits can be destroyed selectively with very little damage to normal intestine (within the light field) by red light activation of the HPD in the tumor initiated 72 hr following a 5-mg/kg i.v. injection of HPD (T. J. Dougherty, unpublished results). Thus, the therapeutic ratio obtained in photoradiation therapy using HPD may depend to a large extent on the particular locale of the tumor under treatment.

Extrapolation of the quantitative tissue levels of HPD in mice to the clinical situation obviously is desirable but unfortunately is not feasible. Our clinical results using HPD plus red light activation to treat a series of cutaneous and s.c. tumors has suggested that human tumors probably accumulate more HPD and retain it for a longer period of time than do the mouse tumors described in this study (3, 5). The serum half-lives for mice and humans are 3 and 25 hr, respectively, following systemic HPD injection (5.0 mg/kg). Thus, we have been able to obtain a very high therapeutic ratio between tumors and exposed normal skin in humans by extending the time period between HPD injection and therapeutic light exposure from 24 hr (as in the mice) to at least 72 hr.

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