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EFFECTS OF HEMATOPORPHYRIN (HPD) AND A CHEMILUMINESCENCE SYSTEM ON THE GROWTH OF TRANSPLANTED TUMORS IN C_3H/HeJ MICE

by

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Photoradiation therapy is emerging as a promising technique for combating cancer. Fundamentally, this approach consists of two steps: (1) hematoporphyrin derivative (HPD) is used to selectively sensitize cancer cells to visible light; (2) after an appropriate time interval, light is introduced into the tumor via a laser-fiber optic system to trigger the cytotoxic action of HPD. The present investigation was initiated to determine the therapeutic potential of HPD in combination with a chemiluminescent activator in treating mice which had been transplanted with tumors.

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20. ABSTRACT CONTINUED

EFFECTS OF HEMATOPORPHYRIN (HPD) AND A CHEMILUMINESCENCE SYSTEM ON THE GROWTH OF TRANSPLANTED TUMORS IN C_3H/HeJ MICE

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ABSTRACT

Photoradiation therapy is emerging as a promising technique for combating cancer. Fundamentally, this approach consists of two steps: (1) hematoporphyrin derivative (HPD) is used to selectively sensitize cancer cells to visible light; (2) after an appropriate time interval, light is introduced into the tumor via a laser-fiber optic system to trigger the cytotoxic action of HPD. The present investigation was initiated to determine the therapeutic potential of HPD in combination with a chemiluminescent activator in treating mice which had been transplanted with tumors.

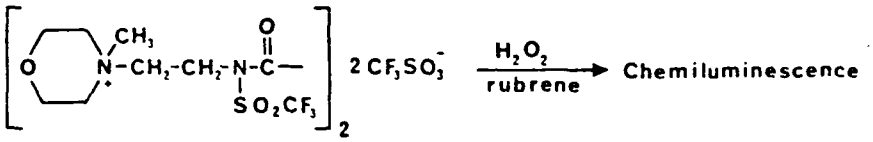
INTRODUCTION

The principles on which photoradiation therapy (PRT) is based have been known for over 80 years. In 1900 it was discovered that certain fluorescent dyes could sensitize living organisms to visible light (Raab, 1900). Some time after this discovery, several investigators reported selective retention of photosensitizing agents by malignant tumors in animals as well as in humans (Auler and Banzer, 1942; Figge et al., 1948; Lipson et al., 1961; Gregorie et al., 1968; Winkelman and Rasmussen-Taxdal, 1969).

More recently, the specific uptake and retention of hematoporphyrin derivative (HPD) by malignant tissue followed by laser irradiation has been utilized in the development of a promising modality for the treatment of

cancer (Kelly et al., 1975; Granelli et al., 1975; Dougherty et al., 1975; Dougherty et al., 1978). Although the mechanism of tumor destruction by PRT has not yet been fully delineated, there is substantial evidence that singlet oxygen is involved as the active agent in the oxidation of biomolecules (Weishaupt et al., 1976; Moan et al., 1979).

Our present investigation was initiated to determine the therapeutic potential of HPD in combination with a chemiluminescent activator. This new approach is targeted at improving the delivery of light to the HPD by replacing the laser with an efficient chemiluminescent system (CLS) that can be injected directly into the tumor. Preliminary results have been obtained in treatment of transplanted tumors in C_3H/HeJ mice.



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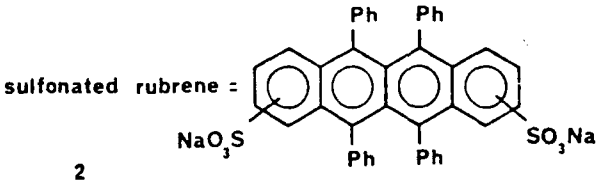

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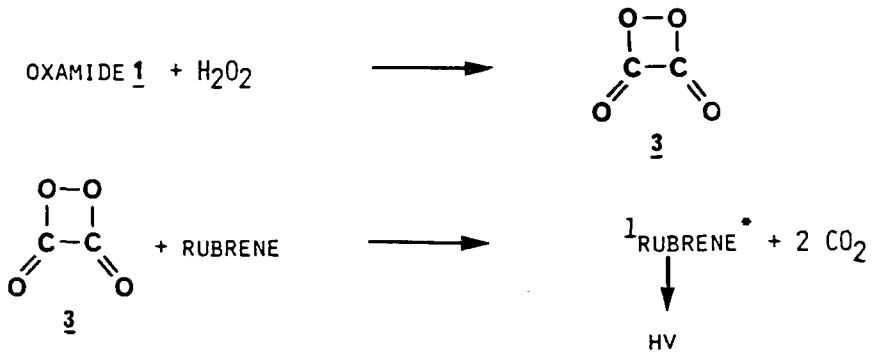
As the chemical light source for this study, we have utilized a system related to the peroxyoxalate chemiluminescence developed at American Cyanamid (Rauhut, 1966; Rauhut et al., 1975; Tseng et al., 1979). The luminescence is produced in aqueous solution by treatment of the substituted oxamide 1 with 1% hydrogen peroxide in the presence of sulfonated rubrene 2 as fluorescer. The reaction is initiated by the addition of the hydrogen peroxide and the surfactant Deceresol N1 to 1 and 2. The intense yellow-red light from this reaction lasts for 10-20 min. The mechanism for this chemiluminescent reaction is thought to involve the formation of the high-energy cyclic peroxide, 1,2-dioxetanedione (3). Subsequent decomposition of peroxide 3 in the presence of rubrene 2 gives singlet excited 2, fluorescence from which provides the observed light.



MATERIALS AND METHODS

A transplanted mammary adenocarcinoma from a female C_3H/HeJ mouse obtained from Henry Ford Hospital was excised after sacrifice of the animal. The tumor was carefully dissected and transplanted into the left axillary fold of 70 C_3H/HeJ male and female mice using the technique described by Phillip et al. (1971, 1973). When the transplanted tumors became palpable, the animals were sensitized with 0.2 mL hematoporphyrin derivative (HPD) obtained from Henry Ford Hospital. The concentration of the HPD was 10 mg/kg body weight. Twenty-four h after sensitization the animals were treated with 0.2 mL of the chemiluminescence system (CLS) described below. The CLS was injected subcutaneously in the area of tumor localization.

Initial samples of oxamide 1, sulfonated rubrene 2, and Deceresol N1 were generously provided by American Cyanamid. We also thank Dr. A. G. Mohan for a description of the procedures for the sytheses of 1 and 2. The chemiluminescence system was prepared by adding 180 mg of oxamide 1, 25 mg of sulfonated rubrene 2, and 0.1 mL of Deceresol N1 to 5 mL of 1% hydrogen peroxide.

RESULTS AND DISCUSSION

This investigation was conducted to evaluate the therapeutic potential of HPD in combination with chemiluminescence. A standardized suspension of viable adenocarcinoma cells excised from a tumor-bearing C_3H/HeJ mouse was

injected into the axillary fold of young healthy mice. A group of animals were given HPD/CLS therapy as soon as the transplanted tumors were palpable. The treatment was administered on two successive days. On the first day the animals were sensitized with HPD and 24 h later the animals were treated with the CLS, injecting directly into the tumor to activate the HPD.

There were four different groups of animals in the investigation. Group I was treated with HPD + CLS; group II was treated with CLS only; group III was transplanted and untreated; and group IV was neither transplanted nor treated and served as a control against the development of spontaneous tumors. The animals in each group were carefully examined at weekly intervals and tumor development was monitored by computing tumor volume.

Average tumor volumes for each group eight weeks after transplantation are shown in Table I. Significantly, group I which was treated with HPD + CLS exhibited approximately four times smaller tumors than the tumors in group III (transplanted and untreated controls). Photograph I shows a typical mouse with a large tumor that was not treated with the chemiluminescence system. In contrast, photograph II demonstrates the typical reduction in tumor volume that is effected by the combined HPD/CLS treatment.

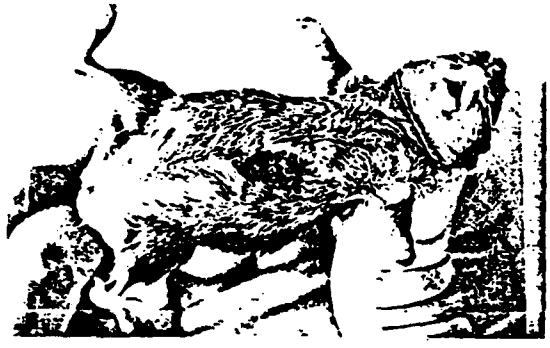
Table I. Average Tumor Volumes Eight Weeks After Transplantation

Group	Volume (cm^3)
I (HPD + CLS)	.6
II (PCS only)	1.4
III (transplanted and untreated)	2.4
IV (normal, not transplanted)	no tumors developed

These results are presently interpreted in terms of a mechanism for tumor destruction involving: (1) localization of HPD in malignant mouse tissue; (2) absorption by HPD of the CLS-produced luminescence; (3) energy transfer from excited HPD to oxygen to generate singlet oxygen; and (4) oxidation of biomolecules in the tumor by singlet oxygen.

Although the results described above are preliminary, we have demonstrated that a chemiluminescent system in combination with HPD may provide an alternate approach to treatment of malignant tumors. This investigation is continuing with studies of various chemiluminescent reactions with other photosensitizing dyes.

Photograph I. Typical mouse with tumor not treated with chemiluminescence system.



Photograph II. Typical reduction in tumor volume upon treatment with HPD and chemiluminescence system.

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