Morphological Study of the Combined Effect of Purpurin Derivatives and Light on Transplantable Rat Bladder Tumors

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ABSTRACT

Purpurin derivatives, a group of synthetic photosensitizers, were tested for their photodynamic activity against transplantable N[4-(5-nitro-2-furyl)-2-thiazolyl]formamide-induced urothelial tumors growing in male Fischer 344 rats. Histological examination of tumors in animals treated with the purpurin derivatives and red light (>=590 nm, 360 joules/cm²) revealed tumor cell necrosis 24 h after completion of therapy. Control tumors showed no histological change.

Purpurins have a major absorption peak in the red region (>=650 nm) of the visible spectrum, a region with good tissue penetration, and purpurins can be synthesized with a high degree of purity. This study demonstrates the potential of purpurin derivatives as photosensitizers for photodynamic cancer therapy.

INTRODUCTION

Photodynamic cancer therapy is based upon the principle that systemically administered photosensitizers localize in neoplastic tissue (1). The accumulation of photosensitizers, as well as the ability to selectively direct visible light onto sensitized tissue, confers a high therapeutic ratio to this form of cancer therapy. It is generally accepted that the combination of visible light and the administered photosensitizer leads to the production of singlet oxygen with the resultant tissue necrosis (2). The most widely studied tumor photosensitizer is HpD, a complex mixture of porphyrins (3). This mixture has an absorption maximum around 405 nm, a wavelength in the electromagnetic spectrum with very limited tissue penetration. A secondary weaker absorption peak exists at 627 nm, a wavelength with better tissue penetration (4). Purpurins, a new group of synthetic photosensitizers, have a major absorption peak between 650 and 715 nm, an area of the visible spectrum with excellent tissue-penetrating properties. In the following study, we investigated whether this group of compounds was photodynamically cytotoxic to transplantable bladder tumors.

MATERIALS AND METHODS

Purpurin derivatives (Fig. 1) NT1, NT2, and NT2H2 were prepared as described previously (5). Purpurin GG1 was prepared following the same procedure except that (carbethoxymethylene)triphenylphosphorane was used in place of (carbethoxymethylene)triphenylphosphorane. ET2 and ET2H2 are analogous to NT2 and NT2H2 except that the starting porphyrin octaethylporphyrin was replaced by acetoporphyrin I. JP1 is analogous to NT2 except that the starting porphyrin was coproporphyrin I tetramethyl ester.

Tumor Model. Transplantable N[4-(5-nitro-2-furyl)-2-thiazoly]formamide-induced urothelial tumors (AY-27) grafted s.c. onto male Fischer 344 rats (Charles River Breeding Laboratories, Boston, MA) were used (6). The necrotic tumor was excised, gently minced in a mixture of Hank's balanced salt solution (Grand Island Biological Co., Grand Island, NY) and antibiotic-antimycotic solution (Grand Island Biological Co.) (penicillin, 10,000 units/ml; fungizone, 25 µg/ml; and streptomycin, 10,000 µg/ml), and passed repeatedly through an 18-gauge needle. The tumor suspension (0.2 ml) was then injected into the s.c. tissue of the abdominal wall of syngeneic animals. Two tumors were implanted into each animal, one below the xiphoid and the other above the pubis. One of the two tumors served as an internal control. Tumors generally became visible within 1 week of implantation and reached 1-cm transverse diameter within 2 weeks.

Purpurin Preparation for Injection. Purpurins are dissolved in a solvent-emulsifier agent Cremophor EL (BASF Wyandotte Corp., Parsippany, NJ). Fifty mg of purpurin are taken up with 1 or 2 ml of warm Cremophor EL depending on the chemical characteristics of individual compounds. The solution was diluted with propylene glycol (1,2-propanediol) to give a final concentration of 32.9% (v/v) with respect to the Cremophor EL. Further dilution with emulsification was achieved by dropwise addition of saline (0.9% sodium chloride), following each addition with vigorous stirring. The final concentration of the drugs was adjusted according to each experimental protocol. For control purposes, the vehicle injections were prepared by diluting 10-fold the solution of Cremophor EL and 1,2-propanediol (1/0, 32.9% v/v) with saline (0.9% sodium chloride). In Fig. 2, the absorption spectra of the purpurin derivative NT2 is shown.

Phototherapy. Animals were anesthetized with sodium pentobarbital (65 mg/kg) during phototherapy. A 500-w G.E. Quartzline lamp (G-GBA; Cleveland, OH) in a Kodak slide projector equipped with a Kodak Ektagan lens (4 in. f2.8) served as a phototherapy unit. The output lens of the projector was fitted with a Corning sharp cut filter (No. 2418; Dow Corning, Corning, NY) to allow only light greater than 590 nm to pass. The light was reflected 90° by placing a 5- x 5-cm silvered mirror 45° to the axis of the light beam, 24 cm from the output lens of the projector. The beam was then passed through a 6-cm diameter double-convex lens with a focal length of 12 cm and focused to give a 1-cm light beam at the surface of the tumor. The light intensity at the tumor surface was measured with a radiometer (UDT No. 351S; Culver City, CA). The tumor temperature was monitored by a 24-gauge hypodermic thermistor probe (No. 524; Yellow Springs Instruments, Yellow Springs, OH) placed percutaneously beneath the surface of the tumor. Core body temperature was monitored with a rectal probe (No. 401; Yellow Springs Instruments). The temperature of the tumors was maintained within 2°C of core temperature (35°C) by a jet of cool air directed over the tumor.

Treatment Protocols. Animals were divided into seven groups of six animals each with each group receiving a different purpurin derivative. Animals were given injections with purpurins (10 mg/kg body weight) under pentobarbital anesthesia (65 mg/kg) via the dorsal tail vein 24 h prior to phototherapy. Injection volumes of the purpurin solutions varied from 0.3 to 0.5 ml depending upon the weight of the animal. One of the two tumors was exposed to 200 mw/cm² of red light through the shaved intact overlying skin for 30 min (360 joules/cm²). The other tumor was shielded from visible light by an opaque box designed to fit closely to the torso of the animal. At 24 h after completion of light treatment three animals in each group were euthanized with an intracardiac injection of saturated KCl and tumors were removed.

Histological Preparation. Tumors were cut into four to five small pieces and fixed in 10% phosphate buffered formalin (pH, 7.2). The fixed tissue was embedded in paraffin, sectioned at 5 µm intervals and stained with hematoxylin and eosin (H&E).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. This work was supported in part by grants from the Geiger Foundation for Cancer Research and the F.M. Douglas Foundation and by the New Investigator Award (NIH R23-CA8754-01).

Received 8/6/86; accepted 10/9/86.

The abbreviation used is: HpD, hematoporphyrin derivative.
PURPURIN DERIVATIVES AS TUMOR PHOTOSENSITIZERS

FIG. A

FIG. B

FIG. C

Fig. 1. Structures of purpurin derivatives.

Fig. 2. Spectrum of injectable solution of NT2 diluted 120-fold.

stained with hematoxylin and eosin. The slides were then examined with a Nikon UFX II microscope fitted with a Nikon FA-35 camera.

RESULTS

In a set of preliminary experiments, it was found that the vehicle alone or in a combination with light had no effect on tumor histology. All of the purpurins tested demonstrated tumoricidal activity when combined with visible light. Control tumors in animals treated with purpurin and no light showed no histological changes. Similarly, shielded tumors in animals treated with purpurin and light showed no histological change (Fig. 3A). Tumors of animals treated with light and NT1, NT2, NT2H2, ET2, or ET2H2 were similar in that they all showed vascular stasis, hemorrhage, and tumor cell vacuolization when examined 4 h after completion of photodynamic therapy (Fig. 3B). Twenty-four hours after completion of light exposure tumors pretreated with these purpurins showed extensive necrosis (Fig. 3C). However, a small rim of histologically viable tumor cells appeared in all examined sections mainly at the periphery of the tumors farthest from the light source (Fig. 3D). Purpurins JP1 and GG1 showed changes similar to those above but the extent of tumor necrosis at 24 h was less extensive. The animals receiving the purpurin injections appeared to suffer no untoward effects from the drugs. No animals treated with the purpurin and light died during therapy.

DISCUSSION

Photodynamic therapy of cancer offers a new method of cancer therapy to the clinician. Photodynamic therapy requires the combination of a photosensitizer and visible light to create the photodynamic effect since either acting by itself is incapable of creating cytotoxicity. To date, the most extensively studied photosensitizer for photodynamic therapy is HpD. This complex mixture of porphyrins is currently undergoing clinical trials to determine its efficacy in the treatment of neoplasms at selected sites. Preliminary studies have reported a response rate of up to 60% (7). HpD has been used because it has the putative property of preferentially localizing in neoplastic tissue. When illuminated with visible light, a photodynamic reaction ensues within the tissues in which the HpD has accumulated. The singlet oxygen which is generated results in tissue destruction. Early studies suggested that HpD accumulated preferentially in neoplastic tissue (8–10). Recent reports, however, indicate that normal tissue can also be destroyed after the systemic delivery of HpD and subsequent illumination with visible light (11, 12). The potential selectivity of this form of therapy may in part lie in the selective application of light. The light source most commonly used for photodynamic therapy is the argon-dye laser system, tuned to deliver red light. Red light has been chosen for HpD photodynamic therapy since it coincides with one of the porphyrin absorption peaks and has greater tissue-penetrating properties than UV light (13).

In spite of the demonstrated efficacy of HpD, there has been an intensive search for new photosensitizers. To be maximally effective these should fulfill the criteria for the ideal photosensitizer which include: (a) should have no systemic toxicity, (b) should be taken up and retained only by malignant tissue, and (c) must absorb light and efficiently destroy malignant tissue at wavelengths not absorbed by normal tissue. Additionally, compound with well-defined chemical structure rather than a mixture should be found. Recently, water-soluble chloroaluminum tetrascufosfopthalocyanine has shown in vivo photodynamic activity (14), while Kessel (15) has shown preferential retention of a hematoporphyrin-chlorin molecule which has a major absorption peak at 650 nm. In vivo, tumoricidal activity has not, as yet, been reported for the hematoporphyrin-chlorin combination.

As opposed to HpD the purpurins used in this study have a well-defined chemical structure. Although they are not water soluble, they have been prepared for injection with a vehicle similar to that used for the delivery of other hydrophobic compounds.

One of the major absorption peaks of the purpurins lies in the far red range of the visible spectrum, a range with excellent tissue-penetrating properties. This should provide improved...
purpurin derivatives as tumor photosensitizers

photodynamic efficiency with the use of these compounds. The histological changes noted in the current studies are very similar to those seen when HpD and light are used to treat the same experimental tumor model (16). The significance of viable-appearing cells 24 h after light treatment is unknown. Whether these cells are capable of further proliferation will be addressed in future studies.

REFERENCES

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