Jejunal Blood Flow after Exposure to Light in Rats Injected with Hematoporphyrin Derivative

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ABSTRACT

Experiments were performed to determine the effect of hematoporphyrin derivative (HPD) photodynamic therapy on blood flow to normal rat intestine. A segment of rat jejunum was exposed to red (>590 nm) light (200 mW/cm²) 24 h after the i.v. administration of HPD. Blood flow to the light exposed segment was determined using the radioactive microsphere technique while blood flow to an adjacent light shielded segment of intestine served as an internal control. Animals were divided into six groups of six each: Group I, no HPD, no light; Group II, light, no HPD; Group III, HPD (20 µg/g body weight), no light; and Group IV, HPD (20 µg/g body weight), light. Blood flow in these four groups was determined 10 min after completion of a 30-min exposure to light. Only in Group IV was there a statistically significant decrease (P < 0.005) in blood flow to the segment treated with HPD and light. In Groups V [HPD (20 µg/g body weight), light] and VI [HPD (10 µg/g body weight), light] blood flows were determined 24 h after exposure to light. In both of these groups there was also a significant (P < 0.05) decrease in blood flow in the segment treated with HPD and light. This study demonstrates that normal intestinal blood flow can be disrupted by HPD photodynamic therapy.

INTRODUCTION

HPD photodynamic therapy is a promising new modality of cancer treatment for a variety of neoplasms (1-3). The use of HPD and light for clinical cancer therapy is based on the assumption that systemically administered HPD is selectively retained in neoplastic tissue and that photodynamic tumor destruction occurs without damage to normal tissue (4, 5). Although the destruction of neoplastic tissue during HPD photodynamic therapy is probably mediated by the production of singlet oxygen (6, 7), the precise mechanism of tumor cell death after photodynamic therapy is unknown. Recently it has become apparent that disruption of tumor blood flow occurs during HPD photodynamic therapy and that this in turn may cause anoxic tumor cell death (8-10). These observations raise the possibility that the abnormal tumor microvasculature could be the target of HPD photodynamic therapy. The following study was undertaken to determine whether the microvasculature of normal tissue was also disrupted by HPD photodynamic therapy.

MATERIALS AND METHODS

Animals. Male Fischer 344 rats (Charles River Breeding Laboratories, Boston, MA) weighing 150-200 g were used in all the experiments. The animals were housed three per cage and fed Purina chow ad libitum. Animals were not fasted prior to experimentation.

HPD Preparation for Injection. HPD (1 g; Porphyrin Products, Logan, UT) was dissolved in 50 ml of 0.1 N sodium hydroxide. The solution was stirred for 1 h at room temperature, neutralized to pH 7.1 with 0.1 N hydrochloric acid, and adjusted to a total volume of 200 ml with 0.9% NaCl solution. Sodium chloride was added to make the final solution isotonic, taking into account the contribution of the sodium salt of the HPD solution. The solution was sterilized by passage through a 0.20-µm Nalgene filter unit (Naïle Co., Rochester, NY). Such solutions are stable for approximately 3 mo when stored in the dark and kept cold (−20°C). HPD was administered by i.v. injection.

Phototherapy Unit. A Kodak slide projector equipped with a 500-W G.E. Quartzline lamp (GE-CBA, Cleveland, OH) and a Kodak Ektanar lens (f4, f2.8) with a Porro prism was used as the phototherapy unit. The light was reflected 90 degrees by placing a 5- x 5-cm silvered mirror 45 degrees to the axis of the light beam, 25 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 serosal surface of the exposed intestinal segment. The light intensity at the serosal surface was measured with a radiometer (UDT No. 351S; Culver City, CA). To determine the power density between the wavelengths of 620 to 640 nm, light power measurements were made using a series of narrow bandpass filters (Oriel Corp., Stanford, CA; Meliss Grits, Irvine, CA).

Technique of Intestinal Phototherapy. Animals were anesthetized with sodium pentobarbital (65 mg/kg b.w. i.p.). Animals in Groups I-IV were anaesthetized with sodium pentobarbital (65 mg/kg b.w. i.p.). Animals in Groups I-IV had left ventricular and femoral artery cannulations performed before intestinal photography (see below). The abdominal wall was then shaved and a midline incision was made. For light treatment, a 3-cm segment of jejunum approximately 5 cm from the pylorus was isolated by passing two 4-0 nylon sutures through the mesentery adjacent to the bowel wall. Care was taken not to injure the mesenteric vessels during this maneuver. These sutures were tied loosely around the intestine in a nonconstricting fashion to aid in identification and suspension of the loop. The light treated intestinal segment was kept on the abdominal wall by gently supporting it with a saline moistened gauze. The remainder of the abdominal viscera as well as the mesenteric of the light treated loop were covered with aluminum foil. The serosal temperature of the exposed loop of intestine was monitored with a 24-gauge hypodermic thermistor probe (No. 524X; Yellow Springs Instruments, Yellow Springs, OH) placed superficially into the serosa. The temperature of the intestine was kept within 2°C of the body core temperature by directing a stream of cool air over the serosa of the bowel and periodically moistening the light treated area with normal saline. The body core temperature was monitored with a rectal probe (No. 401; Yellow Springs Instruments).

Treatment Protocols. Animals were divided into six groups of six each. Animals in groups receiving HPD were given injections 24 h prior to light treatment or sham light treatment. Three control groups were...
used: Group I, no HPD, no light; Group II, light only, 200 mW/cm² for 30 min; Group III, HPD only, 20 μg/g b.w. In Group IV the animals were given injections of HPD, 20 μg/g b.w., and the intestinal segments were treated with light, 200 mW/cm², for 30 min. In Groups I-IV intestinal blood flow was determined 10 min after completion of light treatment. In Groups V (HPD, 20 μg/g b.w.) and VI (HPD 10 μg/g b.w.) the intestinal loop was returned to the abdominal cavity immediately after light exposure (200 mW/cm² for 30 min). The abdominal wall was closed using surgical clips. Twenty-four h later the animals were reanesthetized and blood flow determinations were made.

Regional Blood Flow Determinations. Regional blood flow determinations were made using a modification of the reference sample method described by Malik et al. (11). A tapered PE 50 catheter (Clay Adams, Parsippany, NJ) was advanced into the left ventricle via the right common carotid artery. Continuous monitoring of the arterial pulse pressure via a Statham transducer connected to a polygraph recorder (Beckman Dynograph R511A) confirmed proper placement of the left ventricular catheter as determined by a change in pulse width; catheter placement was subsequently verified at autopsy. Both femoral arteries were exposed and cannulated with PE 10 catheters. The right femoral catheter was connected to a Statham transducer for continuous blood pressure monitoring, while the left femoral catheter was connected to a Harvard syringe drive withdrawal pump. Arterial catheters were filled with a heparinized saline (100 units/liter) after positioning. Microspheres (3.6 × 10³; 15 μm in diameter) labeled with either ¹⁹⁵Ru or ¹⁰³Ce (New England Nuclear, Boston, MA) were injected into the left ventricle over a 10-s period and flushed into the arterial circulation with 1 ml of 0.9% NaCl solution. Microspheres were suspended in 10% dextran containing 0.01% of the surfactant Tween 80, ultrasonicated, and mechanically agitated prior to injection. Starting 10 s before and continuing for 60 s after completion of each injection of the microspheres, a continuous sample of blood was withdrawn from the left femoral artery at a rate of 0.3 ml/min. This sample is referred to as the "reference organ." Animals were then killed by i.v. administration of a saturated solution of KCl.

The light treated jejunal segment and a shielded control intestinal segment 5 cm distal to the treated segment were excised, gently washed with normal saline, cleared of their attached mesenteries, weighed, and placed in scintillation vials for radioactive counting. Kidney, liver, and spleen were harvested at the same time for blood flow determinations. The radioactivity of the reference organ, intestine, and other visceral organs was measured with a Beckman No. 5310 Biogamma Counter.

Blood flow to the intestine (F₁) was calculated from the equation

\[ F₁ = \frac{F_R}{R₁} \times R₁ \]

where \( F₁ \) is the flow to the reference organ, \( R₁ \) is the radioactivity of the reference organ, and \( R₂ \) is the radioactivity of the intestine. Other visceral organ blood flows were determined by substituting radioactivity of the visceral organ for \( R₁ \) in the formula. Blood flow data points are given in ml/min/g and represent the mean ± SE of six blood flow measurements.

Statistical Analysis. Statistical significance was determined by Student's t test for paired values. Calculations were performed on a TI Programmable 59 calculator equipped with a Statistics Library Module (Texas Instruments, Dallas, TX).

RESULTS

As shown in Chart 1 there was no significant difference in blood flow between intestinal segments in Group I (no HPD, no light; 1.633 ± 0.301 versus 1.654 ± 0.506 ml/min/g). In Group II (light, no HPD) there was no significant difference between the light treated segment (1.640 ± 0.142 ml/min/g) and the shielded internal control (1.690 ± 0.163 ml/min/g). Similarly in Group III (HPD, no light) there was no statistically significant difference between the two segments (1.545 ± 0.356 versus 1.552 ± 0.323 ml/min/g). In Group IV (HPD, light) there was a marked decrease in blood flow of the light treated segment (0.146 ± 0.045 ml/min/g) compared to the shielded segment (1.370 ± 0.211 ml/min/g) (P < 0.005). This was also true 24 h after phototreatment in Group V (0.051 ± 0.021 versus 1.408 ± 0.225 ml/min/g; P < 0.002) (Chart 2). In Group VI, in which the dose of HPD was lowered to 10 μg/g b.w., there was also a significantly reduced blood flow to the light treated segment compared to the shielded control (0.151 ± 0.079 versus 1.432 ± 0.197 ml/min/g; P < 0.005).

There was no difference between the blood flow to the right (3.646 ± 0.547 ml/min/g) and left (3.517 ± 0.522 ml/min/g) kidneys reflecting adequate mixing of microspheres. These values are in good agreement with our previously published values for renal blood flow in this animal (9).

DISCUSSION

HPD photodynamic cancer therapy is currently undergoing...
cells and progressed to intravascular stasis. These investigators reported that this might not be the case. Castellani et al. (17) using an autoradiographic study of DBA/2-DA-DO mice bearing the SMT-F mammary carcinoma found a 5-fold grain density ratio in tumor stroma compared to tumor cells after injection of ^3H-HPD. These authors also found endothelial cell damage after HPD photodynamic treatment of these tumors. Additional evidence that the extracellular milieu is an important site of HPD photodynamic activity comes from other studies. Star et al. (10) using transparent observation chambers, showed that tumor blood vessels in transplantable mammary tumors "disappear" after HPD photodynamic therapy. This disappearance was transient but intravascular circulation gradually ceased. Selman et al. (9), using the radioactive microsphere technique to measure blood flow to transplantable bladder tumors, found a rapid and sustained decrease in blood flow after completion of HPD photodynamic therapy. Henderson et al. (8) concluded from tumor cell survival studies using in vivo-in vitro techniques on EMT-6 and RIF experimental mouse tumor systems that damage to tumor circulation was one of the major factors contributing to tumor destruction.

Since tumor vasculature has an abnormal morphology (16), we questioned whether this might be the site of the putative selectivity of HPD photodynamic therapy. Previous studies suggested that this might not be the case. Castellani et al. (17) using hematoporphyrin, the parent compound of HPD, found sequential changes in the microcirculation of the mesentery of the rat and the tongue of the frog, after exposure of these areas to white light. These changes began with agglutination of red blood cells and progressed to intravascular stasis. These investigators phototreated the exposed areas immediately after injection of hematoporphyrin which is dissimilar to current clinical practice in which phototherapy is given 24-72 h after injection of HPD. Gomer et al. (18) documented acute toxicity to normal rabbit retina after HPD photodynamic therapy which correlated with the dose of HPD and light. Fluorescein angiography revealed areas of hypoperfusion and histological examination showed vascular damage after treatment. These changes occurred with light energy densities as low as 36 J/cm^2, 48 h after the administration of HPD, 2.5 mg/kg b.w. Our animals were treated with larger doses of HPD, 20 and 10 mg/g b.w., since we have found previously that doses of HPD less than 10 mg/g b.w. do not induce significant tumor regression in transplantable N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide induced tumors (19). The power output of our phototherapy unit between 620 and 640 nm is approximately 16.2 mW/cm^2. Exposure of the intestinal segment for 30 min to light between these wavelengths results in an energy dose of 33 J/cm^2, well within the range of light energy used in clinical photodynamic therapy (20).

It is clear from the present study that normal intestinal blood flow is disrupted by HPD photodynamic therapy, at least at the doses of HPD and time intervals of treatment used. If the microvasculature is the primary target of HPD photodynamic therapy, then it appears from this and our previous study (9) that both normal and tumor microcirculation are similarly disrupted by photodynamic therapy. From these results it would be difficult to conclude that the selectivity of this form of cancer therapy is attributable to the preferential disruption of tumor blood flow.

REFERENCES


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