Histological Study of the Effect of Hematoporphyrin Derivative Photodynamic Therapy on the Rat Jejunum

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ABSTRACT

Hematoporphyrin derivative photodynamic therapy is evolving as a local treatment for neoplastic disease. The emphasis of previous research has been on the determination of mechanisms of tumoricidal activity and defining the tumoricidal porphyrin component in hematoporphyrin derivative. The effect of hematoporphyrin derivative photodynamic therapy on normal tissue has received little attention. In the following study we examined the morphological changes of normal rat intestine exposed to hematoporphyrin derivative and light. In this model a segment of rat jejunum was exposed to red light (>590 nm; 360 J/cm²) 24 or 72 h after the i.v. administration of hematoporphyrin derivative (5 or 10 µg/g body weight). Control groups received either no treatment, hematoporphyrin derivative only, or light only.

Four h after treatment, intestinal segments were removed and examined by light microscopy. Segments treated with hematoporphyrin derivative and light showed extensive sloughing of the mucosa and submucosa with sparing of the muscular and serosal layers. It appears that hematoporphyrin derivative photodynamic therapy is capable of causing mucosal and submucosal damage to normal rat jejunum at these doses of light and hematoporphyrin derivative.

INTRODUCTION

HpD³ photodynamic therapy is a promising modality for the treatment of selected neoplasms (1). The putative advantage of this therapy lies in the preferential retention of HpD in neoplastic tissue concomitant with its rapid clearance from normal tissues (2). As originally proposed, this form of cancer therapy should cause little damage to normal tissue. In recent studies, however, injury to normal tissue after HpD photodynamic therapy has been reported (3–5). In previous work, we have found a rapid decrease in blood flow to normal intestine after HpD photodynamic therapy has been reported (3–5). In the present study, we describe the histopathological changes occurring in normal rat intestine after its exposure to HpD and light.

MATERIALS AND METHODS

Animals. Male Fischer 344 rats (Charles River Breeding Laboratories, Boston, MA), weighing 150–200 g, were used in all experiments. The animals were housed 3 per cage and were provided with Purina rat chow and water ad libitum.

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 Swinnex-25 filter, 0.45 µm (Millipore Corp., Bedford, MA). Solutions were stored in the dark and kept cold (–20°C). HpD was administered i.v. via the dorsal tail vein in a dose of either 5 or 10 µg/g b.w. The volume of injected HpD was 0.3–0.4 ml³ depending on the weights of the animals.

Phototherapy Unit. A Kodak slide projector, equipped with a 500-W General Electric Quartzline lamp (GE-CBA, Cleveland, OH) and a Kodak Ektanar lens (4 in, f2.8) and fitted with a Corning sharp cut filter (No. 2418; Corning Glass Works, Corning, NY), was used as the phototherapy unit. By reflecting the light beam 90° with a 5- x 5-cm silvered mirror and focusing the beam with a 6-cm-diameter double convex lens (12-cm focal length), an area of 1 cm in diameter at the serosal surface of the jejunum was illuminated with red light (>590 nm). The light was directed at the antimesenteric border of the intestinal segment (see below). The total light intensity for phototherapy was 200 mW/cm² of which the light intensity between 620 and 640 nm is approximately 16 mW/cm². Light intensity was measured with a calibrated photometer/photometer (UDT 5351L; United Detector Technology, Culver City, CA) or an E. G. & G. photometer/radiometer system (Model 450; E. G. & G. Electro-optics, Salem, MA). The light power measurement between 620 and 640 nm was estimated by placing a series of narrow bandpass filters (Oriel Corp., Stamford, CT, and Melles Griot, Irving, CA) into the light beam.

Technique of Intestinal Phototherapy. Animals were anesthetized with an i.p. injection of sodium pentobarbital, 65 µg/g b.w. The abdomen was shaved, and a ventral midline incision was made. A 3-cm segment of jejunum, 5 cm distal to the duodenojejunal junction, was isolated, elevated onto the abdominal wall, and supported with a saline-moistened gauze. A 1-cm segment of the elevated piece of intestine was centered for light treatment with the antimesenteric border closest to the light source. The remainder of the abdominal viscera, as well as the mesentery of the light-treated loop, was covered with aluminum foil. Throughout the duration of phototreatment, the exposed jejunum was kept moist with normal saline to prevent tissue drying. The serosal temperature of the area receiving photoradiation was monitored with a 24 gauge hypodermic thermistor probe (No. 524X; Yellow Springs Instruments, Yellow Springs, OH) placed superficially onto the jejunal surface. The body core temperature was monitored with a rectal probe (No. 40; Yellow Springs Instruments). The temperature of the light treatment area was kept within 2°C of the body core temperature by directing a stream of cool air over the serosal surface.

Preliminary Experiment. Prior to the definitive experiment, a preliminary experiment was undertaken to determine the earliest interval following photodynamic therapy when unequivocal histopathological changes become evident. Animals were given injections of 5 and 10 µg of HpD per g b.w., and intestinal segments were prepared and exposed as detailed above 24 and 72 h following HpD injection. Intestinal segments were examined at 0, 1, 2, 4, and 24 h after light exposure. Maximal damage was evident histologically 4 h after completion of light exposure in these preliminary experiments.

Treatment Protocols. Animals were divided into 7 groups with 3 animals per group. Three control groups were used: Group I, no HpD and no light; Group II, light only; Group III, HpD only, 10 µg/g b.w. Control animals not treated with light underwent laparotomy and intestinal manipulation similar to animals receiving HpD photodynamic therapy (Table 1).

In groups receiving phototherapy, jejunal segments were exposed to red light (>590 nm) (200 mW/cm²) for 30 min. In Groups IV and V, animals were given injections of HpD, 5 µg/g b.w., while in Groups VI and VII, animals were given injections of HpD, 10 µg/g b.w. Following HpD injection, 2 different time intervals were selected for phototherapy. In Groups IV and VI, animals received phototherapy 24 h after...
Gross Morphology. Segments of intestine treated with HpD and light (Groups IV–VII) showed distinctive gross pathological changes. The color of the treated segments was generally a dark brown, and they appeared edematous with multiple filmy adhesions between the treated segments and adjacent loops. Intestinal segments treated with light only appeared slightly edematous, but not discolored, while those treated with HpD only showed no gross changes.

Microscopic Changes. When examined 4 h after phototreatment (Table 1), intestinal segments exposed to light 24 h after HpD injection (5 and 10 µg/g b.w.; Groups IV and VI) showed necrosis, with sloughing of the mucosa and submucosa, throughout the entire circumference of the intestinal wall (Fig. 1D). Only the external muscular layer and the serosa were intact. The intestinal lumina were filled with cellular debris and RBC. When phototreatment was delayed until 72 h after HpD injection in animals that received either 5 or 10 µg/g b.w. (Groups V and VII), there was still histological evidence of extensive change in villi, with necrosis and sloughing; however, the crypts were still present in some areas of the intestinal segments, particularly on the mesenteric border of the intestinal loops (Fig. 1C).

The exteriorized intestinal segments of Group I (no light, no HpD), Group II (light only), and Group III (HpD only) either showed no damage or varying degrees of early inflammation. These changes included focal edema and hyperemia of the villi and the migration of a few polymorphonuclear leukocytes as well as a focal increase in lymphocytes in the lamina propria of the intestines. These changes were maximal in Group II (no HpD, light only) animals (Fig. 1B). The shielded segments in Groups IV–VII showed some similar changes focally.

The number of crypts was not significantly different in Groups I–III. In Groups V and VII, the crypts that could be enumerated were significantly reduced when compared with

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Dose of HpD (µg/g b.w.)</th>
<th>PDT* (&gt;590 nm for 30 min)</th>
<th>HpD-PDT interval (h)</th>
<th>Sampling time after PDT (h)</th>
<th>Histological changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Villi, crypt cells, and outer layers normal</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>+</td>
<td>–</td>
<td>24</td>
<td>4</td>
<td>Edema and hyperemia of intestinal villi</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>10</td>
<td>+</td>
<td>72</td>
<td>4</td>
<td>Edema of some intestinal villi; crypt cells and other layers normal</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>5</td>
<td>+</td>
<td>24</td>
<td>4</td>
<td>Extensive circumferential sloughing of mucosa and submucosa with widespread hemorrhage</td>
</tr>
<tr>
<td>V</td>
<td>3</td>
<td>5</td>
<td>+</td>
<td>72</td>
<td>4</td>
<td>Extensive sloughing of mucosa and submucosa with hemorrhage on the antimesenteric side; submucosa with crypt cells present on mesenteric side</td>
</tr>
<tr>
<td>VI</td>
<td>3</td>
<td>10</td>
<td>+</td>
<td>24</td>
<td>4</td>
<td>Hemorrhagic necrosis and sloughing of mucosa and submucosa circumferentially</td>
</tr>
<tr>
<td>VII</td>
<td>3</td>
<td>10</td>
<td>+</td>
<td>72</td>
<td>4</td>
<td>Destruction of mucosa and submucosa with widespread hemorrhage; presence of surviving crypt cells and submucosa on the mesenteric side</td>
</tr>
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* PDT, photodynamic therapy.

The number of crypts per circumference of the sections was calculated by the method described by Withers (8). The statistical comparison of the various groups was performed by Dunnett's test (14).

<table>
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<tr>
<th>Group</th>
<th>Treatment</th>
<th>HpD-photodynamic therapy interval (h)</th>
<th>No. of crypts/ circumference</th>
<th>Statistical significance (in comparison to Group I)</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>No HpD, no light</td>
<td>138 ± 12</td>
<td>134 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>II</td>
<td>Light only</td>
<td>141 ± 11</td>
<td>0 ± 0</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>III</td>
<td>HpD only</td>
<td>24</td>
<td>85 ± 11</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>IV</td>
<td>HpD (5 µg)</td>
<td>72</td>
<td>57 ± 9</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

* Mean ± SD.
NS, not significant.

Dunnett's test (14). Only the external muscular layer and the serosa were intact. The intestinal lumina were filled with cellular debris and RBC. When phototreatment was delayed until 72 h after HpD injection in animals that received either 5 or 10 µg/g b.w. (Groups V and VII), there was still histological evidence of extensive change in villi, with necrosis and sloughing; however, the crypts were still present in some areas of the intestinal segments, particularly on the mesenteric border of the intestinal loops (Fig. 1C).

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RESULTS

The exteriorized intestinal segments of Group I (no light, no HpD), Group II (light only), and Group III (HpD only) either showed no damage or varying degrees of early inflammation. These changes included focal edema and hyperemia of the villi and the migration of a few polymorphonuclear leukocytes as well as a focal increase in lymphocytes in the lamina propria of the intestines. These changes were maximal in Group II (no HpD, light only) animals (Fig. 1B). The shielded segments in Groups IV–VII showed some similar changes focally.

Table 2

The number of crypts per circumference of the sections was calculated by the method described by Withers (8). The statistical comparison of the various groups was performed by Dunnett's test (14).

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NS, not significant.
EFFECTS OF HpD PHOTODYNAMIC THERAPY ON RAT JEJUNUM

Fig. 1. A, normal small intestine. Villi are delicate, occupying approximately three-quarters of the mucosal thickness. Paraffin embedded, H & E, x 15. B, section of exteriorized portion of small intestine 4 h after exposure to phototreatment without prior administration of HpD. Note the blunting and edema of intestinal villi (arrows). There were a few polymorphonuclear leukocytes, but for the most part, the cells of the lamina propria were lymphocytes which are normally present in this area. Significant acute inflammation was not documented. Similar histological findings were seen in exteriorized segments of Group III (HpD only) and the shielded segments of Groups IV–VII. Paraffin embedded, H & E, x 15. C, exteriorized loop of small intestine in rat receiving HpD (5 µg/g) 72 h prior to light treatment. There is virtually complete necrosis of the mucosal villi. Toward the mesentery there are surviving crypts (arrows). The muscle coat appears intact. The area closest to the light beam showed complete necrosis. Paraffin embedded, H & E, x 15. D, antimesenteric border of exteriorized loop of small intestine, 4 h after phototreatment. The rat received HpD (5 µg/g) 24 h prior to light treatment. There are complete necrosis of mucosal villi and mucosal and submucosal sloughing with only remnants of necrotic villi (arrows). Muscle coat appears intact. The mucosal and submucosal changes were circumferential. Paraffin embedded, H & E, x 15. E, base of crypts from section of normal intestine seen in A above. Note mitotic figures (arrows). x 100. F, base of crypts of HpD and phototreated intestine at 72 h. This corresponds to the area of better preserved mucosa in C above. Note that mitoses are still evident (arrows), x 100.

control groups. In Groups IV and VI, crypts were entirely absent (Table 2).

DISCUSSION

HpD photodynamic therapy is a promising modality, which is receiving increasing application to cancer therapy. The response of experimental and human neoplasms to HpD photodynamic therapy has been well documented (1). However, the response of normal tissue has been less well defined. In this study, we chose small intestine as a model for normal tissue response, because of the ease with which intestine can be exposed to light, the rich intestinal blood supply, the fact that intestinal epithelium is constantly and rapidly being renewed by proliferation of crypt cells, and the lack of intestinal pigmentation, which might complicate interpretation of photosensitivity. Additionally, the intestine has been used in establishing damage from other forms of radiation therapy (7, 8). Among the normal tissues of which the response to HpD photodynamic therapy has been studied, Gomer et al. (3) demonstrated an acute reaction of normal rabbit retina treated with HpD photodynamic therapy. Damage included hemorrhage and retinal detachment. Cheng et al. (4) found extensive damage in normal brain treated with HpD photodynamic therapy. In a clinical trial of patients with esophageal and gastric carcinomas treated with HpD photodynamic therapy, Okuda et al. (5) found not only excellent tumor response, but also extensive necrosis of normal gastric mucosa when light doses exceeded 60 J/cm².

In a time-dependent distribution study of 3H-HpD injected i.v. into rats, Sabben et al. (9) found a low intestinal level of HpD 48 h after injection. From this study one would expect minimal tissue toxicity when phototreatment is delivered greater than 48 h after HpD administration. Douglass et al. (10) suggested from preclinical studies in rabbits that gastrointestinal photodynamic therapy should be limited to 144 J/cm² or less 72 h after HpD (5 µg/g b.w.) injection. Our animals were treated with 200 mW/cm² of red light (>590 nm) for 30 min. However, our light source is noncoherent. The power output of our lamp for a 30-min treatment interval for the wavelengths between 620 and 640 nm is approximately 33 J/
EFFECTS OF HpD PHOTODYNAMIC THERAPY ON RAT JEJUNUM

cm², well within the levels used in clinical photodynamic therapy. Even at these low power levels, considerable mucosal and submucosal damage was found 72 h after HpD injection. The presence of focal histological changes in some segments of intestine from our control animals, in the form of inflammatory changes such as edema, hyperemia, and migration of inflammatory cells, can be attributed to physical injuries, inflicted on the gut during surgical manipulation, or drying that occurs during the photoexposure despite the precautions taken. Scattered light or injury caused by exposure to toxic substances created by HpD photodynamic therapy might also play a role in these changes in segments of intestine at a distance from the treated segment.

A possibly noteworthy histological finding in our experiment is the preservation of crypt cells and their mitotic activity in some areas in groups treated 72 h after HpD administration. The significance of this finding is unclear but might indicate that photodynamic injury is not mediated through injury to the mitotic apparatus as in the X-irradiation (11). In X-irradiation-induced injury, the primary injury of the crypt cells blocks the migration of new epithelial cells to the villi, and eventually the intestinal villi are destroyed. Preservation of cellular division in some crypts following HpD photodynamic therapy suggests that recovery, even from severe damage, may be possible. This will be further investigated.

In previous studies, we have documented a disruption of blood flow to N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide-induced transplantable urothelial tumors treated with HpD and light (12). This has been corroborated by other studies, and it has been suggested that anoxia caused by a decrease in tumor blood flow may in part explain the tumoricidal action of HpD photodynamic therapy (13). We have recently investigated the response of normal intestinal blood flow to HpD photodynamic therapy and found that HpD photodynamic therapy resulted in a rapid cessation of intestinal blood flow (6). Whether the histological changes documented in this investigation were the result of ischemia or direct cytotoxicity will be the subject of further investigation.

REFERENCES

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