Colonic Mucosectomy Using Laser Photodynamic Therapy

D. GARTH FISHER, M.D., ERIC B. RYPINS, M.D., LUKE R. WATSON, M.D.,
J. STUART NELSON, M.D., PH.D., AND MICHAEL W. BURNS, PH.D.
Departments of Surgery and Pathology, University of California, Irvine Medical Center,
Orange, California 92668; and The Beckman Laser Institute, Irvine, California 92715

Presented at the Annual Meeting of the Association for Academic Surgery, Salt Lake City, Utah, November 16-19, 1988

Photodynamic therapy (PDT) involves photosensitizing tissue and then activating it with monochromatic light, causing necrosis. Precise control of the extent of injury should be possible by varying the energy density of the light applied to the target tissue. We tested the sensitivity of colonic tissue to PDT by injecting 10 mg/kg Photofrin II intraperitoneally in 10 rats. After 24 hr the left colon was opened and cleansed. A 1.0-cm² area of mucosa was exposed to 630 nm (red) light produced by an argon-pumped dye laser. Pairs of rats were treated with energy densities of either 10, 20, 40, 60, or 80 J/cm², controlled by varying exposure times. After 48 hr, we sacrificed the rats and fixed, sectioned, and stained the left colons. The depth of injury was measured with an ocular micrometer and expressed as a percentage of normal bowel wall thickness. A curve was fit to the data points by computerized nonlinear regression. The relationship between depth of injury (Y) and energy density (X) was found to fit the equation Y = 1 - ae⁻bx, where constants a = 1.15 and b = -0.0353, (R² = 0.93, P < 0.001). The relationship between injury and energy density is biphasic, rising rapidly from 0 to 40 J/cm² and more slowly after this point, suggesting that colonic mucosa is more sensitive to PDT than muscularis, providing a margin of safety against perforation. Bowel perforation did not occur in this study but is predicted by extrapolation for energy densities of 100 J/cm² or greater. These data indicate that photodynamic colonic mucosectomy is possible.
610–690 nM. The dye laser was tuned to emit radiation at 630 nM for the entire experiment. The wavelength was verified to ±1 nM using a Jobin Yvon No. 5/354 ultraviolet monochromator (Longjuneau, France). The radiation was coupled into a 400-μm fused silica fiber optic using a Spectra Physics (Mountain View, CA) model 316 fiber optic coupler. The output end of the fiber was terminated with a microlens that focused the laser irradiation into a circular field of uniform light intensity. Laser irradiation emanating from the fiber was monitored with a Coherent (Palo Alto, CA) Model 210 power meter before, during, and after treatment. Rats were placed underneath an aperture that controlled the area of light illumination on the colon. The area of illumination was 1 cm². Total laser energy density was 10 to 80 J/cm² with a power density of 100 mW/cm².

The five pairs of rats were treated with energy densities of 10, 20, 40, 60, or 80 J/cm² respectively, controlled by varying exposure times. The colons were then closed with running 5-O proline and the abdominal incisions were closed with silk sutures. After 48 hr the rats were sacrificed with Euthasix injection. The laser-treated segments of colon were excised and placed in randomly numbered vials of saline. As a result of a laboratory accident, one colon specimen treated with 40 J/cm² was lost, so nine specimens were available for examination. The specimens were examined grossly and were then stained with hematoxylin and eosin. A pathologist, blinded as to the dose of laser energy applied to the bowel, examined the mounted specimens for both gross and microscopic changes. In addition, using an ocular micrometer, the depth of injury was measured as well as the full-thickness of adjacent intact bowel wall. The degree of injury was expressed as a percentage of the full thickness wall, allowing for comparisons between rats.

The depth of injury was plotted against the energy density delivered to the bowel in joules per square centimeter. The curve was analyzed on an IBM-AT computer using nonlinear regression (NONLIN program, Statgraphics Software, STSC, Inc., Rockville, MD). An equation relating depth of injury to energy density was generated by the program. An analysis of variance (ANOVA) for the full regression is performed with an associated $P$ value and $R^2$ statistic.

FIG. 1. Photomicrograph (50X) of colon wall after exposure to 10 J/cm² laser energy. The injury is about 25% of bowel wall thickness or 50% of the mucosal thickness.
FIG. 2. Photomicrograph of colon wall after exposure to 20 J/cm². The entire mucosa is ablated with mild fibrosis and preservation of the muscularis mucosa. Mild to moderate inflammation was present in the submucosa.

FIG. 3. Photomicrograph showing injury to colon exposed to 40 J/cm². There is full thickness loss of mucosa and muscularis mucosa with inflammation and edema of the submucosa.
FIG. 4. Photomicrograph showing injury to colon exposed to 60 J/cm². Besides the findings described for the energy density of 40 J/cm², there is also thinning and focal loss of the submucosa.

FIG. 5. This photomicrograph shows the extensive injury found after exposure to 80 J/cm². There is total absence of mucosa, muscularis mucosa, and submucosa. The muscularis propria is thinned and focally absent. The ulcer base extends to the serosa but perforation is not seen.
RESULTS

Sections of colon receiving 10 to 40 J/cm² were grossly normal in appearance with retention of normal mucosal folds. Colons treated with 60 and 80 J/cm² showed obvious mucosal ulceration. Microscopically, injury to the mucosa was evident at the lowest energy density studied. An energy density of 10 J/cm² ablated 50% of the mucosa thickness (Fig. 1). There was minimal lymphocyte infiltration of the underlying lamina propria. Sections of colon receiving 20 J/cm² developed full thickness loss of the mucosa (Fig. 2) but the muscularis mucosa remained intact. There was mild to moderate acute inflammation throughout the adjacent submucosa. In the section of colon receiving 40 J/cm², there was total loss of the mucosa and muscularis mucosa (Fig. 3). The submucosa was moderately edematous and infiltrated with acute and chronic inflammatory cells. Bowel receiving 60 J/cm² was ulcerated, with absence of mucosa and muscularis mucosa (Fig. 4) and thinning of the submucosa. There was a moderate amount of fibrosis and acute inflammation with fibrinopurulent exudate throughout the submucosa. Colons exposed to 80 J/cm² (Fig. 5) were more deeply ulcerated with total absence of mucosa, muscularis mucosa, and submucosa extending to, and focally through, the muscularis propria. The serosa remained intact across the ulcer base. Throughout the muscularis propria and subserosa there was marked inflammation and a fibropurulent exudate. However, free perforation of the bowel did not occur.

The depth of injury related to the energy density is shown graphically in Fig. 6. The nonlinear relationship between injury and energy density is readily apparent. This relationship is biphasic, rising rapidly from 0 to 40 J/cm² (through the mucosa) and more slowly after this point. The curve best fit an exponential equation, \( Y = 1 - ae^{bx} \) \((R^2 = .93, P < 0.001)\), where \( Y \) is the depth of injury, \( a \) and \( b \) are constants 1.15 and \(-0.0353\), respectively, and \( X \) is the energy density in J/cm².

DISCUSSION

Photodynamic therapy uses a photosensitizing dye that is selectively absorbed by target tissues. The dye is activated by monochromatic light where it causes an intracellular photochemical reaction that liberates singlet oxygen and kills the cell. Unlike most laser methods familiar to surgeons, this method does not use heat to ablate tissue and so avoids much of the risk commonly associated with laser destruction of tissue.

Normal colonic mucosa selectively absorbs Photofrin II, hindering its usefulness for treating colon carcinoma [4]. However, in using Photofrin II for sensitizing normal colon mucosa, we have exploited this property for a different end, ablating the normal mucosa. Is photodynamic therapy practical and safe for mucosectomy? The biphasic nature of the injury–energy curve indicates that colonic mucosa is more sensitive to phototherapy than muscularis. This finding suggests a margin of safety against perforation. While bowel perforation did not occur in this study. The curve predicts by extrapolation that perforation will occur for energy densities greater than 100 J/cm². This study shows that laser photodynamic therapy could possibly be a practical way of ablating colonic mucosa.

REFERENCES

1. Goligher, J. C. Eversion technique for distal mucosal proctectomy in ulcerative colitis: A preliminary report. Brit. J. Surg. 71: 26, 1984.
2. Kojima, Y., Sanada, Y., and Fonkalsrud, E. W. Evaluation of techniques for chemical debridement of colonic mucosa. Surg. Gynecol. Obstet. 155: 849, 1982.
3. Dougherty, T. J., Kaufman, J. E., Goldfarb, A., Wcislo, K. R., Boyle, D., and Mittleman, A. Photoradiation therapy for the treatment of malignant tumors. Cancer Res. 38: 2628, 1978.
4. Berns, M. W., Wilson, M., Rentzepis, P., Burns, R., and Wile, A. G. Cell biology of hematoporphyrin derivative. Laser Surg. Med. 2: 201, 1983.
5. Dahlman, A., Wile, A. G., Burns, R. G., Mason, G. R., Johnson, F. M., and Berns, M. W. Laser photoradiation therapy of cancer. Cancer Res. 43: 430, 1983.
6. Agrez, M. V., Wharen, R. E., Jr., Anderson, R. E., Laws, E. R., Jr., Ilistrup, D. M., Cortese, D. A., Shorter, R. G., and Lieber, M. M. Hematoporphyrin derivative: Quantitative uptake in dimethylsulfoxide-induced murine colorectal carcinoma. J. Surg. Oncol. 24: 173, 1983.
7. Woodard, T., Black, K., Knapp, R., and Achauer, B. M. Anesthesia in the microsurgery laboratory. J. Microsurg. 3: 61, 1981.