Gynecology

Photodynamic destruction of endometrial tissue with topical 5-aminolevulinic acid in rats and rabbits

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OBJECTIVE: The goal of this study was to determine the optimal parameters for photodynamic endometrial destruction with topically applied 5-aminolevulinic acid, a precursor for the endogenous synthesis of the fluorescent photosensitizer protoporphyrin IX.
STUDY DESIGN: 5-Aminolevulinic acid pharmacokinetics were measured in rat and rabbit models by analyzing tissue frozen sections 3 to 12 hours after topical administration. Dose-response studies were conducted for 100 to 400 mg/ml 5-aminolevulinic acid. Photodynamic therapy was performed intraluminally, and tissue morphologic features were evaluated 3 and 7 days after treatment.
RESULTS: Peak fluorescence was observed 3 hours after topical administration. Glandular fluorescence significantly exceeded stromal and myometrial in all studies, particularly for 200 mg/ml 5-aminolevulinic acid. Histologic studies revealed persistent epithelial destruction with minimal regeneration.
CONCLUSION: Topical 5-aminolevulinic acid photodynamic therapy can be used for highly effective, long-lasting destruction of endometrial epithelium. However, optical dosimetry can vary, particularly in the rabbit model, and this appears to have an impact on long-term reepithelialization. (Am J Obstet Gynecol 1994;171:1176-83.)

Key words: Photodynamic therapy, endometrium, 5-aminolevulinic acid, protoporphyrin IX, pharmacokinetics

The use of photodynamic therapy as a tool for the destruction of endometrial tissue[1-4] and endometriotic implants[5-6] has generated considerable interest in the past few years. Possible targeting of endometrial tissue and simple light delivery may facilitate this approach to clinical application. Photodynamic therapy generally uses intravenous administration of a photosensitizing drug that is selectively retained by target tissues. Light activation results in the generation of highly reactive oxygen intermediates.[7] These intermediates, primarily singlet molecular oxygen, irreversibly oxidize essential tissue and cellular components.[8] The resulting photodestruction ultimately causes local injury and necrosis.

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Prolonged skin photosensitivity is an unwanted side effect of intravenously administered photosensitizers; however, it can potentially be avoided by topical application. Because the anatomy of the female genital tract allows direct access to the uterine cavity, we have pursued the development of endometrial photodynamic therapy with topically administered 5-aminolevulinic acid.

5-Aminolevulinic acid is a precursor of protoporphyrin IX in the biosynthetic pathway of heme and occurs in aerobic cells. The slowest process in heme synthesis is the protoporphyrin IX to heme conversion. Therefore administration of exogenous 5-aminolevulinic acid induces the accumulation of protoporphyrin IX, which is a potent photosensitizer.[9]

Endometrial destruction after topical administration of 5-aminolevulinic acid has been performed successfully in rats with water-based 5-aminolevulinic acid solutions.[4] It is well known that low-viscosity (e.g., aqueous) fluids pass easily through the fallopian tubes into the abdominal cavity. To minimize this effect before human application, we studied the pharmacokinetics of 5-aminolevulinic acid in dextran 70 (Hyskon, Kabi Pharmacia, Piscataway, N.J.), a viscous, hydrophilic, branched polysaccharide used for uterine distention during hysteroscopy.

In a previous study Yang et al.[4] evaluated reproduc-

tive performance in rats after photodynamic therapy with 40 to 160 mg/ml 5-aminolevulinic acid. Our study was designed to explore the distribution and pharmacokinetics of 100 to 400 mg/ml 5-aminolevulinic acid–Hyskon in rat and rabbit uteri. 5-Aminolevulinic acid solutions ≥40 mg/ml are highly acidic; when injected into the uterine cavity they may cause local damage and painful contractions from prostaglandin $F_{2\alpha}$ release.[10] As a result, we discuss the impact of pH adjustment on 5-aminolevulinic acid–induced protoporphyrin IX synthesis in various uterine layers and assess photodynamic therapy–induced structure damage by means of both light and scanning electron microscopy.

Material and methods

Animals. Twelve mature female Sprague-Dawley rats weighing 270 to 395 gm and 42 mature female New Zealand White rabbits weighing 3600 to 4300 gm were placed in a controlled environment with free access to food and water. Twelve rats and 12 rabbits were contributed to the pharmacokinetic study to determine uptake and distribution of protoporphyrin IX by fluorescence microscopy. Twenty-four rabbits were added to study the relationship between drug concentration, pH, and relative fluorescence. Six rabbits were used to evaluate histologic changes after photodynamic therapy. Pharmacokinetic studies with 5-aminolevulinic acid–Hyskon solutions in rats were performed on the day of diestrus (the estrous cycle was monitored by vaginal cytologic study), to focus on the glandular and stromal cells. Glandular proliferation occurs only during diestrus, stromal during diestrus and proestrus, and myometrial during proestrus.[11] Additionally, there is no detectable intrauterine fluid during diestrus influencing the drug solution. Rabbits are induced ovulators; therefore no estrous monitoring was required.

Photosensitizer. Crystallized 5-aminolevulinic acid hydrochloride (Deprenyl USA, Parsippany, N.J.) was stored in the dark at a temperature of 4° C and diluted to 100, 200, 300, or 400 mg/ml in Hyskon shortly before administration. The injected volume was 0.15 ml in rat and 1.2 ml in rabbit uterine horns, respectively (left side only). To minimize Hyskon dilution, 5-aminolevulinic acid solutions were titrated with 10N and 1N sodium hydroxide to pH 5.5.

Procedures. Animals were anesthetized with ketamine/xylazine (2:1), 0.75 ml/kg given intramuscularly, for both animal species, and isoflurane was added during the surgical intervention. Intrauterine drug application was performed through a midline incision. The photosensitizer was injected with a 20-gauge needle into the left horn 3 to 5 mm distal to the uterine bifurcation. Abdominal walls were closed in two or three layers (Dexon 4-0 staples, Davis & Geck, Inc., American Cyanamid Co., Manati, P.R.) in rats and rabbits, respectively. Temperature, pulse, and respiration were monitored until the animal was ambulatory. Uterine specimens were retrieved by a second laparotomy immediately after death (rats: asphyxiation with carbon dioxide gas; rabbits: intracardiac injection of 1.5 ml Euth-6 [Western Medical Supply, Arcadia, Calif.] after isoflurane anesthesia). Specimens were sectioned in four blocks of 3 to 4 mm each and placed in molds containing embedding medium for frozen sections (ornithine carbamyl transferase media, Miles, Elkhart, Ind.). The blocks were rapidly frozen on dry ice and stored at −70° C in the dark. Specimens retrieved for histologic examination were fixed in 10% formaldehyde.

5-Aminolevulinic acid pharmacokinetics was evaluated by analyzing frozen sections from rats and rabbits killed 3, 6, 9, and 12 hours after administration of 100 mg/ml 5-aminolevulinic acid–Hyskon. A dose-response study was conducted for 100, 200, 300, and 400 mg/ml 5-aminolevulinic acid–Hyskon in rabbits at the time of maximum protoporphyrin IX fluorescence (3 hours, determined from the pharmacokinetic study). Three animals were studied for each time point and drug dose. To determine the effects of pH adjustment, identical dose-response studies were conducted in rabbits with untitrated 5-aminolevulinic acid–Hyskon (pH range 1.6 to 2.2).

Photodynamic therapy was performed on six rabbits with intrauterine illumination. Light from an argon-pumped dye laser operating at 630 nm (Spectra Physics, Mountain View, Calif.) was delivered to the uterine cavity with a 400 μm diameter quartz optical fiber terminated with a 3.0 cm cylindric diffusing tip (model 4420-A02, PDT Systems, Buellton, Calif.). A clinical Hartridge Reversion spectroscope (Ealing Electro-Optics, South Natick, Mass.) was used to verify the wavelength. Specimens were retrieved at 3 or 7 days (three animals each time point) after photodynamic therapy for light and scanning electron microscopy. Because the length of the rabbit uterine horn was 12 to 15 cm, multiple (four to five) segmental irradiation was required. A total of 255 mW was launched into the fiber (85 mW/cm per fiber tip) during 1200 seconds, resulting in a variable tissue dose that, depending on geometry, ranged from 80 to 160 J/cm^2.

Fluorescence microscopy. The frozen specimens were sectioned in low diffuse light (Cryostat microtome, AO Reichert, Buffalo, N.Y.) at 6 μm slices. Low-light-level tissue fluorescence imaging was performed with a slow-scan, thermoelectrically cooled CCD camera system (model ST-180, Princeton Instruments, Trenton, N.J.) coupled to a Zeiss Axiovert 10 inverted fluorescence microscope (Carl Zeiss, Inc., Oberkochen, Germany). A 10× objective (Zeiss Planneofluar [numeric aperture 0.3]) was used to visualize bright-field and fluorescence images of the tissue frozen sections. A 100 W mercury arc lamp coupled to a mechanical shutter

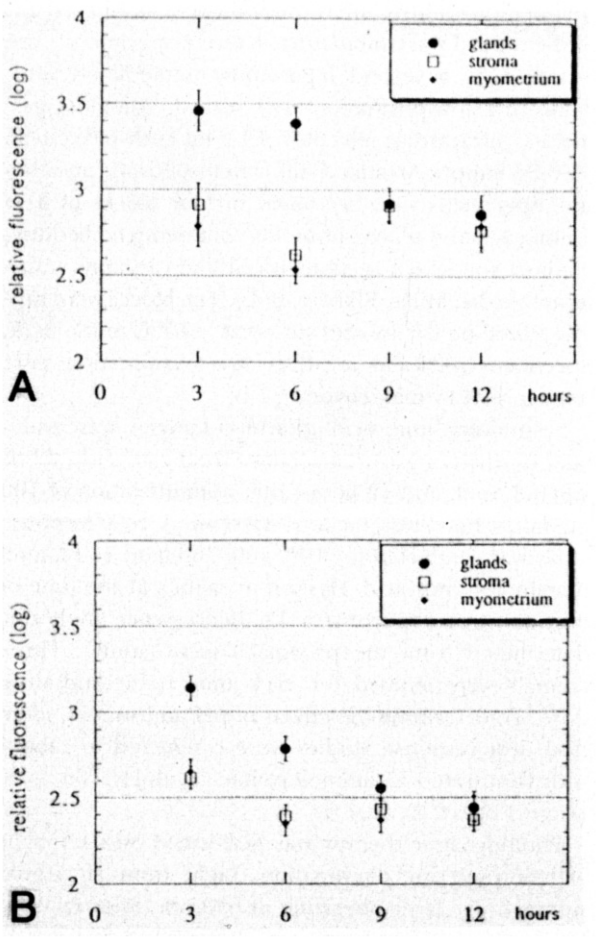

Fig. 1. Log protophorphyrin IX fluorescence in glands, stroma, and myometrium versus time for rats **(A)** and rabbits **(B)** determined from fluorescence microscope images of tissue frozen sections (three animals per time point).

(UniBlitz model T132, Vincent Associates, Rochester, N.Y.) and filtered through a 405 nm, broad-bandpass filter provided excitation light. A dichroic mirror (FT 420) reflected the excitation light onto the sample and transmitted the fluorescence emission through a 635 nm broad-bandpass filter onto the detector.

Instrument control, image acquisition, and processing were performed with a MacIntosh IIfx computer and IPlab software (Signal Analytics, Vienna, Va.). Minimal sample photobleaching was achieved by synchronizing mercury lamp and charge coupled device camera shutters. To estimate light distribution, background images were acquired from blank slides with identical parameters. All fluorescence images were normalized by the following algorithm to correct for nonuniform illumination:

$$\text{Normalized fluorescence image} = \frac{\text{Mean (Background} - \text{Dark noise)}}{\text{Image (Background} - \text{Dark noise)}} \times \text{Image (Fluorescence} - \text{Dark noise)}$$

Table I. Concentration-dependent pH

5-Aminolevulinic acid (mg/ml)	*Acidity (pH)*
100	2.2
200	2.0
300	1.8
400	1.6
Hyskon	4.7

Both fluorescence and background images were corrected for dark noise contributed during the exposure time.

Rat and rabbit uteri were divided into different anatomic layers for comparative analysis. Measured compartments were endometrial glands, endometrial stroma, and the circular muscle (myometrium).

Histologic study. Samples for histologic study (light microscopy and scanning electron microscopy) were fixed in 10% formalin in phosphate buffer at room temperature for 24 hours. Light microscopy samples were dehydrated in graded ethylene alcohol, cleared in Histo-Clear (National Diagnostics, Manville, N.J.) infiltrated with paraffin by means of a tissue processor (model 155MP, Fisher Scientific, Pittsburgh), and embedded in paraffin. Sections were cut at 6 μm, deparaffinized, and stained with either hematoxylin and eosin or sirius red 3BA. Scanning electron microscopy specimens were fixed as above and further processed in 10% osmium tetroxide, dehydrated in graded acetone, critical point dried (Ladd Critical Point Dryer, Ladd Research Industries, Burlington, Va.), and sputter coated with gold (Pelco PAC-1 evaporating system, Ted Pella, Redding, Calif.). Micrographs were then taken on scanning electron microscopy (SEM 515, Philips Electronic Instrument Company, Mahwake, N.J.).

Data analysis. All statistical analyses were performed on the logarithmic transformation of fluorescence data, which was done to homogenize the variability of the groups being compared. Multiple fluorescence measurements from individual animals were averaged. At each time point (3, 6, 9, and 12 hours) the average of three animals was calculated (total 12 rats and 12 rabbits). Four variables were examined: fluorescence of muscular, stromal, and glandular tissues and difference in fluorescence between stroma and glands. To assess the effect of time on the four variables, two analyses were performed. First, linear regression methods were used to test a linear trend (rats and rabbits); second, Student *t* test was used to compare results obtained at 3 and 6 hours with those at 9 and 12 hours (rats). Finally, the difference in glandular and stromal fluorescence was evaluated with a paired Student *t* test (rats and rabbits). Means and SEs were also calculated for fluorescence data of different concentrations. Repeated-measures analysis of variance was used to examine the

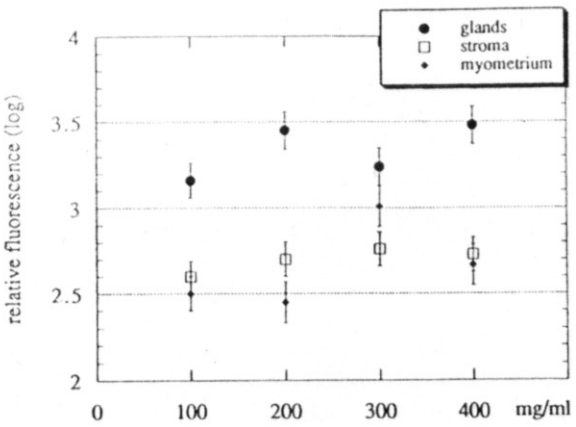

Fig. 2. Log protoporphyrin IX fluorescence in glands, stroma, and myometrium versus 5-aminolevulinic acid dose (milligrams per milliliter) for rabbits determined from fluorescence microscope images of tissue frozen sections (three animals per 5-aminolevulinic acid dose).

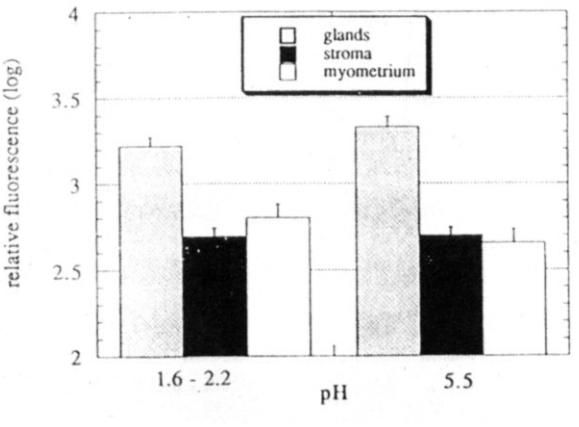

Fig. 3. Log protoporphyrin IX fluorescence in glands, stroma, and myometrium for untitrated and pH-adjusted 5-aminolevulinic acid–Hyskon (12 animals each group). Data were obtained from fluorescence microscope images of tissue frozen sections prepared for dose-response studies.

difference in fluorescence with concentration. The Student *t* test was also used to compare the fluorescence of untitrated and titrated animals with data for all concentrations.

Results

Pharmacokinetics. Fig. 1, *A* and *B*, shows higher protoporphyrin IX fluorescence in glands than stroma and myometrium for all times. Peak fluorescence, and hence protoporphyrin IX conversion, was observed at 3 hours in both animal models. There was a similar uptake pattern between rats and rabbits in the endometrial glands. A significant linear decrease in the fluorescence of glands was noted across time in rats and rabbits ($p = 0.001$ and $p = 0.05$). There was a significant decrease in rabbit stroma fluorescence ($p = 0.05$) as well but not in rat stroma fluorescence. No linear trend was seen for circular (myometrium) tissue in both species. Overall, the glandular fluorescence exceeded that of the stroma with nine of 10 rats ($p = 0.01$) and with all 12 rabbits ($p = 0.001$). In rats the difference is greater at 3 and 6 hours (mean difference 0.67) than at 9 and 12 hours (mean difference 0.04), which is highly significant ($p = 0.0006$). In rabbits the difference decreased with time ($p = 0.0006$), from an average difference of 0.52 at 3 hours to 0.07 at 12 hours.

To evaluate dose response, uterine structure fluorescence was measured after topical administration of 100, 200, 300, and 400 mg/ml 5-aminolevulinic acid–Hyskon solutions. Fig. 2 summarizes these dose-response data for pH 5.5 5-aminolevulinic acid–Hyskon. No significant dose-dependent fluctuations were observed in glandular, stromal, and myometrial fluorescence when concentration of the 5-aminolevulinic acid was increased from 100 to 400 mg/ml. However, glandular uptake was significantly higher than that of the other structures regardless of drug concentration ($p = 0.0001$). Fluorescence contrast between glands and stroma-myometrium varied significantly with dose ($p = 0.0042$), ranging from a peak difference of 1.00 for glands-myometrium at 200 mg/ml to 0.23 at 300 mg/ml.

Dose-response data for untitrated and pH-adjusted 5-aminolevulinic acid–Hyskon are summarized and compared in Fig. 3 (untitrated dose-response not shown). These results clearly demonstrate that there were no significant fluorescence variations within uterine layers after pH adjustment. Table I shows that concentration-dependent pH variations for 5-aminolevulinic acid–Hyskon were observed to range from pH 2.2 to 1.6.

Histologic studies after photodynamic therapy. Micromorphologic changes after photodynamic therapy were evaluated in the right (untreated) and left (treated) uterine horns of the same rabbit with both light and scanning electron microscopy. Light microscopy results are shown for ×100 and ×16 magnifications in Fig. 4. Scanning electron microscopy images (×5000) are presented in Fig. 5.

Low-magnification light microscopy images of untreated (Fig. 4, *A*) and treated (Fig. 4, *B*) uterine horns clearly show destruction of luminal and glandular epithelial structures 7 days after photodynamic therapy. The endometrial stroma remains largely intact with moderate scarring. Minimal reepithelialization is occasionally observed in some sections (for example, upper right, Fig. 4, *B*), perhaps because of uneven light dosimetry. High-magnification images (Fig. 4, *C*, *D*) revealed dramatic changes in the columnar epithelium

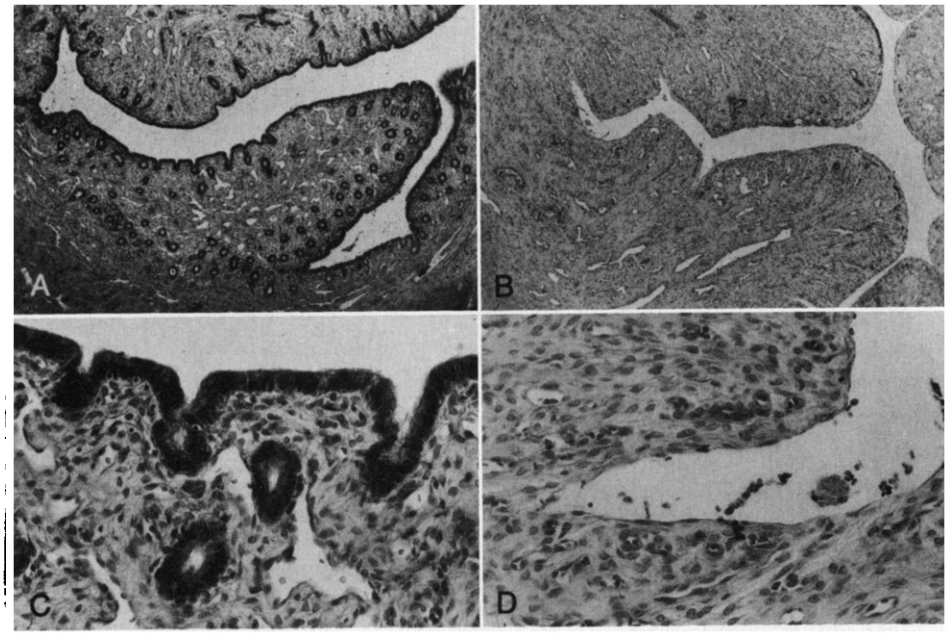

Fig. 4. Light microscopy images of untreated **(A, C)** and treated **(B, D)** uterine horns 7 days after photodynamic therapy. (Original magnification: **A** and **B**, ×16; **C** and **D**, ×100.)

and the structural integrity of glandular invaginations. In addition, the postirradiation luminal surface is composed of elongated, flattened fibroblasts and extracellular matrix components, whereas the lumen contains fibrinous and cellular debris. Three-day-postirradiation findings (not shown) are comparable and, in some cases, more acute with indications of hyperemia.

Scanning electron micrographs of the untreated uterine horn (Fig. 5, *A*) show ciliated cells surrounded by nonciliated microvillous cells. In contrast, the treated horn (Fig. 5, *B*) exhibits pronounced superficial damage. No evidence of the normal epithelial structure remains, and the surface appears to be replaced by a collagen network resembling scar tissue or, perhaps, basal membrane.

Comment

Previous studies in rats[1, 4] and rabbits[2] suggest that photodynamic therapy may provide an effective alternative to conventional techniques (e.g., curettage, electrosurgery, and laser photocoagulation) for selective endometrial destruction. This minimally invasive approach could benefit patients with dysfunctional uterine bleeding and possibly endometrial neoplasia by minimizing the frequency of traumatic surgical interventions. However, several complex, interrelated factors including photosensitizer type, concentration, and delivery mode (systemic vs topical) and light dose, delivery, and timing ultimately determine the success or failure of endometrial photodynamic therapy. These general parameters must be well characterized in model systems to optimize the probability of success in humans.

In this report we describe our efforts to develop a simple, rapid technique for endometrial ablation with topical 5-aminolevulinic acid in both rat and rabbit animal models. Pharmacokinetic investigations confirm previous observations that protoporphyrin IX synthesis is primarily glandular (unpublished data) and peaks approximately 3 hours after topical application. Overall, glandular fluorescence significantly exceeds stromal levels, indicating selective targeting of epithelial structures. It is interesting to note that this is probably one of the most dramatic differences between 5-aminolevulinic acid and the more established photosensitizer Photofrin (Quadra Logic Technologies, Inc., Vancouver), because topical application of the latter results in nearly identical glandular and stromal fluorescence (unpublished data). Glandular dominance with 5-aminolevulinic acid is probably related to differences in cellular metabolism and, fortuitously, may provide selectivity during irradiation. Light dose timing is also important for selective destruction, and our pharmacokinetic data suggest that 3 hours is the optimum drug-light interval. However, because all irradiations were performed at 3 hours, we did not explicitly evaluate this parameter.

The influence of drug dose on protoporphyrin IX synthesis was evaluated by several different 5-aminolevulinic acid concentrations (100 to 400 mg/ml). These dose-response studies indicate that, in the case of pH 5.5 5-aminolevulinic acid–Hyskon, there are no significant concentration-dependent variations in protopor-

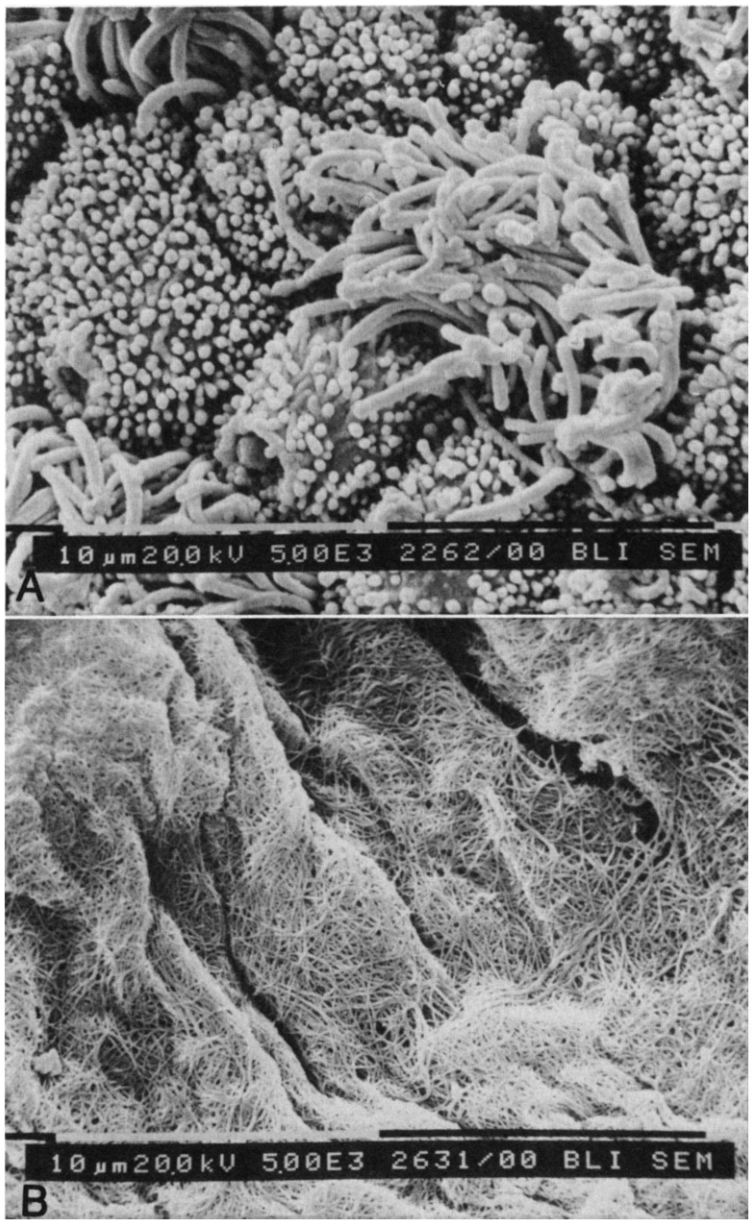

Fig. 5. Scanning electron micrographs of untreated **(A)** and treated **(B)** uterine horns 7 days after photodynamic therapy. (Original magnification ×5000.)

phyrin IX production. Glandular fluorescence is uniformly elevated regardless of 5-aminolevulinic acid concentration. However, substantial differences between glandular, stromal, and myometrial fluorescence appear at the 200 mg/ml dose. Accordingly, we conducted all irradiation studies 3 hours after topical application of 200 mg/ml 5-aminolevulinic acid–Hyskon.

In the course of the dose-response studies we observed concentration-dependent pH variations ranging from pH 2.2 at 100 mg/ml 5-aminolevulinic acid–Hyskon to pH 1.6 at 400 mg/ml. Because the pH of uterine fluid in humans during the various phases of the menstrual cycle ranges from 5.9 to 7.3,[12] we were concerned that acidic 5-aminolevulinic acid solutions applied to the uterine cavity might be toxic to the endometrium. Low pH exposure results in endogenous prostaglandin $F_{2\alpha}$ release,[10] which, in turn, can cause painful uterine contractions, spillage of photosensitizer, and a decrease in uterine blood flow. Low blood flow can reduce tissue oxygen saturation and, as a result, influence singlet oxygen generation during photodynamic therapy.[13] In contrast, alkaline solutions (pH ≥7.4) did not cause contractions in rats.[14] It is interesting to speculate that the highly acidic 5-aminolevulinic acid solutions used in previous studies may, in part, account for the slight reduction in the number of

implanted embryos observed for nonirradiated control uteri.[4]

Because of the potentially deleterious effects of low pH, all photodynamic treatments were performed with 5-aminolevulinic acid–Hyskon adjusted to pH 5.5. This value was selected because titration to pH ≥ 6.0 leads to turbidity and, ultimately, an intense yellow solution that may have a different physiologic behavior from the more highly protonated acid. Results of a comparison study indicate that pH adjustment does not alter fluorescence levels or protoporphyrin IX synthesis. In addition, our Hyskon-based data are similar to those previously reported for water and saline solution.[4] Hyskon is a clear, viscous, hydrophilic branched polysaccharide used for uterine cavity distention hysteroscopy. It was selected in an effort to minimize retrograde spillage through the cervix and passage through the fallopian tubes into the abdominal cavity. Although we have no conclusive evidence that Hyskon enhances 5-aminolevulinic acid availability, there is no indication that Hyskon adversely affects 5-aminolevulinic acid delivery to the target tissue.

Our findings from the pharmacokinetic, dose-response, pH, and Hyskon studies were used to determine optimal parameters for photodynamic destruction. Photodynamic therapy was conducted with 200 mg/ml, pH 5.5 5-aminolevulinic acid–Hyskon 3 hours after topical 5-aminolevulinic acid application. Light (630 nm) was delivered intraluminally by a 3 cm cylindric diffusing fiber tip. A total of 255 mW was launched into the fiber (85 mW/cm fiber tip) during 1200 seconds, resulting in a tissue dose that varied with lumen geometry. Although we estimate the dose ranged from 80 to 160 J/cm^2, the actual dose is nearly impossible to determine because there are substantial local variations in cavity size and shape. In addition, the 12 to 15 cm rabbit uterine horn required multiple[2, 15] segmental irradiation. Consequently, there may have been regions that received inordinately high or low light doses. Furthermore, differences in fiber location with respect to mucosal folds can lead to an inhomogenous light field. These factors can influence both the rate of destruction and, because of photobleaching, the amount of available photosensitizer.

In spite of these complex dosimetry factors the degree of destruction was consistent between animals evaluated 3 days after irradiation. However, it is important to point out that, after 7 days, epithelial regeneration varied with anatomic location. We selected a 7-day end point because regeneration after mechanical destruction of the endometrium in rabbits is complete after 3 days.[16] Although our 7-day observations generally revealed substantial, persistent destruction (for example, Figs. 4 and 5), regional variations in reepithelization are a clear indication that maintaining the optical dose at or above the photodynamic threshold is critical to obtaining irreversible damage. Drug distribution may also play a role in these findings; however, our dose-response data indicate that there is no real practical value to increasing the drug dose beyond the 200 mg/ml level used for photodynamic therapy. As a result, it is expected that photodynamic therapy after topical application of 5-aminolevulinic acid can be used for highly effective endometrial destruction. We have explored a variety of factors intended to optimize this procedure; however, the prospect of long-term tissue regeneration is a possibility and should be evaluated in more rigorous light-dose and light-delivery studies.

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Evaluation of tumor-associated glycoprotein-72 and CA 125 serum markers in patients with gynecologic diseases

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OBJECTIVE: This study was performed to evaluate the clinical values of tumor-associated glycoprotein-72 serum levels alone or in combination with CA 125 in the diagnosis and monitoring of patients with ovarian cancer.

STUDY DESIGN: Serum samples from 293 patients, 142 with primary carcinoma and 151 with benign diseases of the genital tract, were evaluated for the presence of CA 125, tumor-associated glycoprotein-72, and carcinoembryonic antigen. All patients underwent surgery for the primary tumor, and stage was defined according to the classification of International Federation of Gynecology and Obstetrics.

RESULTS: When the measurement of serum tumor-associated glycoprotein-72 is combined with that of CA 125, the sensitivity for the detection of primary ovarian cancer increased from 60% to 73%, with no significant change in specificity, and resulted in a more accurate clinical assessment for detection of residual disease before the second-look procedure. In fact, when both markers were positive, 100% specificity was achieved; conversely, when both markers were negative, no residual disease was found.

CONCLUSION: These findings suggest that tumor-associated glycoprotein-72 may be considered as a supplementary serum marker for CA 125, providing further clinical information for the diagnosis of primary and recurrent ovarian cancer. (Am J Obstet Gynecol 1994;171:1183-91.)

Key words: Tumor-associated glycoprotein-72, CA 125, ovarian cancer

Ovarian cancer is a frequent cause of death from cancer and the leading cause of death from gynecologic cancer in industrialized countries.[1] The disease is characterized by long latency periods, which most often results in clinical diagnosis at an advanced stage. Current clinical management involves cytoreductive surgery followed by combination chemotherapy, which has resulted in longer disease-free periods and improved survival rates. In addition, second-look procedures (i.e., laparotomy or laparoscopy) are also part of the follow-up of patients suspected of having recurrent ovarian disease.[2] Recent developments in ultrasonography and the identification of novel serum tumor markers may provide new means of monitoring patients suspected of having ovarian carcinoma, evaluating the response to treatment, or diagnosing recurrent disease.[3, 4]

CA 125 has been the most widely studied human tumor antigen associated with ovarian carcinoma. An initial report[5] revealed the presence of elevated CA 125 levels in 83% of serum samples obtained from patients with surgically demonstrable epithelial ovarian cancer. Subsequent studies revealed that 25% of the patients with nongynecologic malignancies had elevated serum CA 125 levels.[6] Only 1% of healthy blood donors[5, 7] and 5% of patients with benign disease had elevated CA 125 serum levels (cutoff >35 U/ml).[5] Nevertheless, more

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