Photodynamic therapy towards selective endometrial ablation.

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ABSTRACT

Potential applications of Photodynamic therapy for endometrial disease is discussed. Experimental models that may lead for diagnosis and treatment of endometriosis as well as selective endometrial ablation are summarized.

Photodynamic therapy (PDT) is an experimental technique used in the treatment of certain tumors [1]. The process typically involves intravenous administration of a photosensitizing drug that is retained longer in certain malignant and vascularaized tissue. When light of sufficient energy and appropriate wavelength interacts with the sensitizer, highly reactive oxygen intermediates are generated. These intermediates, primarily singlet molecular oxygen, irreversibly oxidize essential cellular components. The resulting photodestruction of crucial cell organelles and vasculature ultimately causes tissue necrosis [2]. In addition to their therapeutic effect, the characteristic red fluorescence exhibited by photosensitizers can be used in tissue diagnostics. By recording the spatial and spectral distribution of drug fluorescence and tissue autofluorescence, high -contrast images of malignancies can be acquired Endometrial tissue (in situ or ectopic, i.e. endometrium, adenomyosis and endometriosis) may well serve as both an experimental model and a target tissue for PDT. It is a highly vascular, neo-proliferative tissue with selective sensitivity to hormonal stimulation.

Schneider et al [3] studied the potential use of PDT for selective endometrial ablation in rat uteri. The same group further evaluated the influence of estrogen on the uptake and localization of dihematoporphyrine ether (DHE) in the uterus of ovariectomized rats [4]. The photosensitizer was concentrated in the endometrial tissue and estrogen treatment significantly increased the uterine uptake but had no effect on other organs. Endometrial ablation by means of PDT with Photofrin in the rabbit was recently described[5]. The drug was injected I.V. (1,2,5 and 10 mg/kg) and 24 hr later, I.U. laser illumination at 630 nm

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was administered. The authors concluded that endometrial ablation can be effectively achieved in rabbits by means of PDT.

The potential of PDT for the treatment of endometriosis was evaluated by Manyak et al[6]. The authors induced endometriosis surgically by transplantation of endometrial tissue in female rabbits. The animals were injected (I.V.) with DHE and 24 hours later, transplants were exposed to 630 nm light. Complete and selective epithelial destruction was seen in 60-81% of the animals with direct relationship to the light dose. Endometrial implants were surgically induced for PDT also by Petrucco et al [7]. Gold vapor laser irradiation (operating at 627.8 nm) of the transplanted area, pretreated with hematoporphyrin derivative [HPD], produced necrosis of the endometriotic lesions, leaving surrounding tissue healthy.

To improve endoscopic diagnosis, Vancaillie et al [8] studied the laser induced fluorescence of ectopic endometrium in a similar rabbit model. Photosensitive drugs were used following surgery and estrogen stimulation, however, as opposed to other studies they used photoenhancers with some specific estrogen affinity. Laser irradiation of the experimentally induced ectopic endometrium failed to induce fluorescence except with the administration of the photoenhancers. Tamoxifen and clomiphen induced intense fluorescence at the normal endometrium in situ, however, only tamoxifen induced fluorescence of the peritoneal endometrial implants.

Using a different model, Manyak et al[9] induced early stage endometriosis by intraperitoneal injection of monodispersed viable endometrial cells. They found that DHE fluorescence facilitates detection of endometrial tissue.

Kennedy studied the fluorescence following systemic administration of a different photosensitizer, 5 Aminolevulinic Acid (ALA), in the mouse uterus[10]. The endometrium became strongly fluorescent, whereas the myometrium did not. Yang (personal communication) in a recent study have clearly demonstrated that intrauterine injection of ALA followed by light exposure caused persistent and specific photodynamic ablation of rat endometrium. In a recent presentation, Judd et al, [11]have studied fluorescence of the uterine layers following I.V. injection of ALA and Phthalocyanine. The endometrium showed a peak fluorescence at 2 and 3 hours with ALA and Phthalocyanine respectively. When using the ALA, the endometrial layer showed fluorescence levels 5 times higher than the myometrium.

The response of human endometrial carcinoma (HEC-1-A cell-line) and ovarian carcinoma (OvCar-3) to PDT in vitro was studied by Raab et al [12]. Both cell lines did not survive PDT. Complete ovarian cell death was observed after application of irradiation doses in the range of 5-20 J/cm^2 combined with drug concentrations of 2.5-10

μg/ml at fixed incubation of 48h. The endometrial cells did not survive PDT with 10 J/cm^2 after incubation in 5 μg/ml for 48h.

In order to better understand the determinants of selective uptake and retention of various photosensitizers in uterine tissue, we have systematically investigated this topic in rat models [phases I-II] [13]. The main questions we addressed in phase one of these studies were: a) what is the preferred mode of drug application, and b) what is the influence of estradiol (as an endometrial proliferation stimulator) on the selectivity and duration of drug uptake.

Since Photofrin is currently the most commonly used photosensitizer, this compound was the main drug employed. Initially, we evaluated the relative merits of intravenous (IV), intraperitoneal (IP), and intrauterine (IU) administration methods in medically or surgically castrated rats. Extraction of Photofrin from uterine tissue was conducted according to a modified porphyrin fecal extraction technique[14]. Frozen sections were analyzed by fluorescence microscopy. In order to characterize the distribution of drug fluorescence in uterine layers. Histologic specimens were fixed in 10% buffered formalin at room temperature for 24 hours and then washed in phosphate buffer, dehydrated in graded alcohol, cleared in histoclear and embedded in paraffin. Using a histomatic tissue processor, 7 μm sections were cut, deparaffined and stained with hematoxylin & eosin.

Intraperitoneal Photofrin administeration resulted in a definite pattern of uptake and redistribution within the uterus, as well as a higher overall uptake than with the IV approach. This trend suggested that initially there was a high concentration of Photofrin in the serosa, however as time elapsed the drug moved towards the endometrium. Again, myometrial uptake and retention persisted up to 48 hours, although it was not significantly higher than IV delivery. It is not clear whether this redistribution was due to diffusion or absorption into the vascular system and subsequent redistribution.

Intrauterine (IU) delivery of the photosensitizer appeared to allow for more selective retention within the surface endometrial cells (over all time intervals) and minimized myometrial uptake.

On the basis of fluorescence intensity it was determined that the drug remained within the surface endometrial glands with limited diffusion into the deeper stromal layers. Uptake by the endometrial stroma was not significantly different at 48 hours as compared to IV administration. However, the relative distribution favored uptake within the endometrium with limited uptake by the myometrium. The elevated mitotic activity and increased protein production within the surface endometrial cells and deeper stromal cells may have increased the concentration and retention of the drug in these two layers.

Finally, despite a 10-fold reduction in dose, IU application yielded a significant increase

in extracted Photofrin, lending support to the hypothesis that site specific delivery of the photosensitizer can result in selective retention of the drug at a much reduced dose.
In a subsequent experiment[13], all rats were administered the photosensitizer by IU application. The main question was focused on the effect of estrogen on uptake and retention of the photosensitizer within the uterine layers and other control organs. Serum estradiol levels were determined by direct radio immunoassay (the minimum detectable estradiol concentration was 10 pg./ml.). Fluorescence activity within the surface of endometrial glands was most prominent in the estradiol stimulated rats. There was some fluorescence in the deeper stromal cells, with all groups showing some pockets of bright fluorescence. Photofrin, however, tended to be excluded from the myometrial layer especially following estrogen stimulation. This appeared to be due to the presence of an active, thicker endometrial layer. There are possible explanations for the prolonged retention of Photofrin within the epithelium. As a relatively hydrophobic compound , once it is inside the epithelial cellular lining, it binds to the metabolically active components within the cytosol (recently stimulated production by Estradiol [E2]). If the endometrium has been recently stimulated by E2, stromal cells and endometrial glands will exhibit prolonged binding of the Photofrin . The distribution pattern of fluorescence within the endometrial stroma may reflect products from the breakdown of Photofrin within the surface endometrial cells that diffuse into the stroma, while still retaining their fluorescent and photodynamic properties.
Although E2 stimulation yielded equivocal increases in fluorescence in the endometrial layer (columnar epithelium and stroma), it did substantially increase the overall amount of porphyrins extracted from the uterus. Thus, E2 stimulation appears to result in greater photosensitizer uptake. There were no significant E2-induced changes in the uptake of the drug in control organs (spleen and thigh muscle).
Our preliminary studies combined with the limited information available in the literature encouraged further investigation of this approach. Although the IU application of drug appeared promising, drawbacks to these studies included the relatively high Photofrin concentration in the columnar epithelium and the absence of data correlating our drug distribution and studies with photodynamic efficacy. ,A penetration-enhancing agent (Azone [1-dodecylazacycloheptane-2-one] was added to Photofrin, and a similar rat model was used to compare fluoerscence distribution in uterine layers following topical application of Photofrin to Photofrin with Azone.
It was clearly demonstrate that Photofrin with Azone penetrates much faster than Photofrin alone to the deeper endometrial layers.

Further studies are under way to define the optimal conditions for selective endometrial ablation. It is expected that photodynamic destruction of endometrial tissue might replace surgical procedures that require general anaesthesia and hospitalization. PDT has the potential to improve the conventional management of endometrial disease and minimize the cost and invasiveness of treatment.

Table 1. summary of endometrial photosensitization studies.

Author	Model	Photo-sensitizer	Dose (mg/kg)	Laser -WL	Aim
Schneider[6]	**Rat -** *Endometrium*	**DHE-** IV	7	630	Endometrial Ablation
Schneider[4]	**Rat -** *Endometrium*	**DHE-** IV	7	630	Effect of Estrogen
Vancaillie[8]	**Rabbit** *(Surg. Induced Endometriosis)*	I.M. - **Clomiphene Tamoxifen Tetracycline**	- X2 / day 5mg/1ml 10mg/1ml 20mg/1ml	351.1 and 363.8nm Argon Ion (Fluores.)	1. Photoradiation diagnosis of endometriosis with various photoenhancers.
Manyak[9]	**Rabbit -** *(Surg. Induced Endometriosis)*	**DHE - IV**	10	630	1. Endometriosis treatment. 2. Laser parameters
Petrucco [7]	**Rabbit -** *(Surg. Induced Endometriosis)*	**HPD - IV**	50	629.7	1. Endometriosis treat. 2. Tissue Fluorescence.
Manyak [9]	**Rabbit -** *(Endometrial Cell Dispersion)*	**DHE - IV**	10	366 Fluores.	1. Endometriosis Diag. & treatment.
Raab[12]	**Human** * *Endometrial Ca.*	**Porphyrin** (In Vitro)	0-10 µg/ml medium	630	* In vitro response of endometrial cancer to PDT
Bhatta [5]	**Rabbit -** *Endometrium*	**Porphyrin**	1,2,5, 10	630	Endometrial Ablation
Judd [11]	**Rabbit -** *Endometrium*	**ALA - IV**	200	/	Compare Tissue Fluorescence-uptake.
Kennedy 10	**Mouse-** *Uterus*	**ALA - IV**	N/A	/	Compare Tissue Fluorescence-uptake.
Yang (personal communication)	**Rat-** *Endometrium*	**ALA - IU**	4,6,16, (mg/ 0.1ml)	Non-laser. Red light	Endometrial ablation
Chapman [13]	**Rat-***Endometrium*	**DHE:** - IV - IP - IU -	7 - 7 - 0.7	/	*Pharmacokinetics,: Fluorescence study of various applications*
Chapman [13]	**Rat-***Endometrium*	**DHE** - IU	0.7	/	*Pharmacokinetics,: -Fluorescence study -Influence of E2*

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