Urol Res (2001) 29: 205–209

ORIGINAL PAPER

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Effect of photodynamic therapy on urinary bladder function: an experimental study with rats

Received: 10 April 2000 / Accepted: 16 November 2000

Abstract Photodynamic therapy (PDT) produces localized necrosis with light after prior administration of a photosensitizing drug. The problems with laser light dosimetry and complications relating to bladder function appear to be important limiting factors of PDT in urology. Photodynamic therapy on urinary bladder with normal epithelium of rats was performed using an argon ion laser as an energy source, with aminolevulinic acid (ALA)-induced protoporphyrin IX (PpIX) photosensitizer. Four hours after ALA intravenous administration, the bladders were intravesically radiated with light doses 20, 40, or 80 J/cm^2. Animals in the control group did not receive ALA and were radiated with 20 J/cm^2 light dose. Three weeks prior to PDT, the bladder capacity and pressure changes during filling cystometry were assessed. Cystometrics were repeated 1, 3, 7, or 21 days after laser therapy. The light dose 20 J/cm^2 and 40 J/cm^2 together with the used ALA dose caused no reduction in bladder capacity, whereas 80 J/cm^2 light dose produced up to 50% reduction in the capacity at 3 weeks postoperatively. In control group without ALA, the animals did not regain more than 34% of the capacity of their control values at 3 weeks. The light dose of 20 J/cm^2 and 40 J/cm^2 with ALA induced functional changes that subsided after day 1. Our results indicate that with proper dosing of photosensitizing drug and light energy, the functional impairment of urinary bladder may be reduced as transient. These findings support the use of PDT as safe therapy of superficial bladder cancer.

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Key words Photodynamic therapy · Aminolevulinic acid · Urinary bladder · Functional changes

Introduction

The treatment of urologic malignancy was one of the earliest clinical applications for PDT ever since interest was regenerated for this modality in the 1970s. Kelly et al. first reported urologic use of PDT in 1975, with the destruction of human transitional cell carcinoma transplants in mice [7]. PDT uses an interaction between an absorbed light and a photosensitizer in the presence of oxygen in order to destroy tissue. The relative selective retention of photosensitizer within or around tumor cells may allow more selective destruction of neoplasm and limit damage to adjacent or systemic normal tissues [12].

The tissue photosensitizer protoporphyrin IX (PpIX) is an immediate precursor of heme in the biosynthesis pathway for heme. The rate of synthesis of PpIX is determined by the rate of synthesis of 5-aminolevulinic acid (ALA), which in turn is regulated via a feedback control mechanism governed by the concentration of free heme. Exogenous ALA bypasses the feedback control, and thus may induce the intracellular accumulation of photosensitizing concentrations of PpIX. The resulting tissue-specific photosensitization provides a basis for using ALA-induced PpIX for photodynamic therapy [8].

Unfortunately, it is difficult to produce truly selective tumor necrosis with PDT based on selective uptake of the sensitizer, and the incidence of complications may be significant. Irritative urinary symptoms are common but usually transient, generally resolving within 2 weeks [17, 21]. Deeper bladder wall destruction like muscle fibrosis and permanently reduced bladder capacity, incontinence, and upper tract deterioration are reported with symptoms which may themselves necessitate cystectomy [6, 17, 21]. The troublesome side effects of PDT on the bladder appear to be due to necrosis in the muscle layer of the bladder. It would be expected that if the tissue

concentration of photosensitizer was below the threshold in the muscle but above it in the mucosa, then there might be a mechanism for limiting PDT effects to the superficial layers [20]. Aminolevulinic acid-induced PpIX appears to localize preferentially in the mucosa rather than the muscle of hollow organs, and accumulation in the rat bladder is highest by 4 h after ALA administration [2, 5, 10, 11, 13].

However, it is now becoming apparent that the nature of the biological effects of PDT on normal and malignant tissues is different from that produced by other forms of local injury, such as thermal coagulation or ionizing radiation [19]. Tissue architecture and tensile strength are much better preserved after PDT than after thermal injury and PDT has little effect on collagen whereas heat destroys it [1].

The wide range of light doses used in clinical series of PDT for superficial bladder cancer is remarkable [4, 7, 14, 16, 18, 19]. To avoid irreversible functional impairment in the bladder, it is essential to select correct light and sensitizer doses. There are only a few studies concerning the functional changes in the bladder after PDT. Without basic experiments on the exact mechanism of PDT in the normal bladder, it is no doubt too complicated to treat bladder cancer in humans with PDT. The aim of this study was to find out the proper light dose to induce only a transient disturbance in bladder function after laser therapy.

Materials and methods

Animals

For this study, 64 female Wistar rats, each weighing approximately 200 g, were obtained from the National Laboratory Animal Center (University of Kuopio). The animals were divided into three PDT therapy groups: laser dose 20 J/cm^2, 40 J/cm^2, and 80 J/cm^2. The animals receiving only laser radiation with dose 20 J/cm^2 served as controls. Each therapy group was divided into four groups, with four animals in each, according to the date of cystometric analysis, after which they were killed. During the time between ALA-injection and laser therapy, the animals were kept in subdued lighting as a precaution against potential skin photosensitization. All animals studies were approved by the Ethics Committee of the University of Kuopio.

Laser equipment

A Spectra-Physics Model Stablite 2016 continuous-wave argon-ion laser served as a pumping source for a Spectra-Physics water-cooled argon-dye laser. The generally accepted absorption maximum of PpIX in the red is at 635 nm, but PpIX absorbs light up to 700 nm. DCM ([2-[2-[4-(dimethylamino)phenyl]ethanyl]-6-methyl-4H-pyran-4-ylidene]-propanedinitrile) dye lasered from 638 to 650 nm, and it was not possible with this laser device to obtain any lower wavelengths. These wavelengths were measured with a Kratos Model GM 100-1 monocromator driven by a Kratos GMA Motor drive. A Spectrum was output to Servogor type SE 120 plotter. The pumping source had a maximum output power of 4 W, but the actual maximum power was 1.6 W (measured with a laser power measurement system, Scientech Mentor MAIO indicator and Scientech Mentor MC 2500 calorimeter). Losses due to absorption and reflections of dye, mirrors and resonator, fiber junction, and fiber itself decreased the maximum power to 250 mW, measured at the fiber tip. The fiber used was a 200 μm optical fiber with a ball-shaped tip (diameter 300 μm).The irradiation times varied from 4 min 49 s up to 19 min 16 s, dependent on the totally applied light dose. The laser equipment was installed on Zero-G vibration isolation Systems table and so the measurements were carried out at high stability.

Chemicals and aminolevulinic acid administration

Aminolevulinic acid as the hydrochloride (Sigma Chemical, UK) with a purity of 98% was prepared immediately prior to intravenous administration. It was dissolved in physiological saline and administered in a volume 0.3 ml at a dose 300 mg/kg. The animals were anesthetized by subcutaneous injection of a mixture of phentanylfluanisone (Hypnorm, Janssen Pharmaceutica) and midazolam (Dormicum, Roche) and weighed. Aminolevulinic acid solution was injected into the tail vein via a 25 G needle. The animals in the control group did not receive ALA.

Photodynamic therapy

The animals were sensitized with ALA (300 mg/kg) 4 h prior to light exposure, as suggested by our previous fluorescence study, to achieve the optimum concentration ratio between the mucosa and muscle layers of the bladder wall [5]. For infection prophylaxis, all animals were given 15 mg benzylpenicillinprocain (Ilcocillin 300 mg/ml, Orion-Farmos) subcutaneously. For PDT, the rat bladder was catheterized with a 20 G cannula and filled to a volume of 0.3 ml with 0.02% soybean emulsion (Intralipid) in saline to achieve the proper diffusion medium for isotropic light distribution in the bladder. The bladder was assumed to be spherical in calculations for light energy-dosing. The bladder was exposed through a lower abdominal incision. The urethral cannula was removed, and the 200-μm optical fiber with a ball-shaped tip was positioned centrally within the bladder via the urethra. Laser power of 150 mW was used with lasering times of 4 min 49 s, 9 min 38 s, and 19 min 16 s, to achieve total energy doses of 20, 40, and 80 J/cm^2. Control animals without photosensitizer received only 20 J/cm^2 light. In the three treatment groups, all animals received 300 mg/kg ALA intravenously and were illuminated with different light doses.

The temperature of the bladder walls during PDT was monitored in all animal groups to exclude thermal effects. The temperature was monitored from the outer surface of the bladder by placing the thermometer in the central part of the bladder anterior wall. The difference in temperature measured before and after laser irradiation was 1.0 °C in the control group and in the groups of 20 J/cm^2, 40 J/cm^2, and 80 J/cm^2 light with ALA 2.3 °C, 1.8 °C and 2.4 °C, respectively.

Filling cystometry measurement

For each animal, filling cystometry was performed during the 3 weeks before PDT and after treatment at days 1, 3, 7, or 21, according to grouping. Each animal served as its own control. For cystometry, the rat bladder was cannulated with a 20 G Venflon R (BOC Ohmeda, USA) cannula. This was connected via a three-way tap to an invasive pressure measurement transducer (Braun, Germany) and to a syringe pump. The lines were rinsed with normal saline and carefully checked to exclude all air bubbles in the line. To minimize flow resistance in the lines, the system was calibrated to zero at the beginning of bladder filling. The pressure measurement transducer was connected to an AS/3R (Datex, Helsinki, Finland) intensive care monitor, which was connected to a Toshiba T 1000 PC that collected the measured data at a sample rate of 3 s to a file. A constant infusion rate of 0.2 ml/min was maintained with a syringe pump. Physiological saline kept at room temperature was used as filling fluid. As end points of cystometric measurement the following criteria were used: 2 ml infused volume or

16.3 cm H_2O filling pressure or uninhibited bladder contractions seen as a visible urine leakage from the urethra.

Statistical analysis

The measured values are presented as means ± SEM. Differences in intravesical pressure between the control and test groups were assessed using the Mann-Whitney U-test. Results were considered statistically significant at $P < 0.05$.

Results

Filling cystometry

In pretreatment analysis, the intravesical pressure increased gradually with infused volume and reached 8–11 cm H_2O level. The infused volumes varied from 1.2 ml to 2.0 ml saline of room temperature. In some cases, urine leakage was observed also in lower pressure values, and fluid infusion was stopped immediately (Fig. 1).

Animals which received only 20 J/cm^2 light without ALA served as control. At day 1, the cystometry was similar to pre-treatment measurements, but in later measurements (3, 7, and 21 days) the bladder pressure was significantly increased. Even at 21 days after treatment, the bladder pressure was higher than in all PDT treatment groups ($P < 0.05$) and the change to pre-treatment measurement was significant ($P < 0.05$).

Animals receiving light doses 20 J/cm^2 and 40 J/cm^2 together with ALA had a similar behavior. In both groups at day 1, the pressure showed a sharp rise ($P < 0.05$), but returned to pre-treatment levels before day 7 and stayed there at day 21.

Those animals which received the highest light doses, 80 J/cm^2, did not recover during the 21 day follow-up period from PDT therapy. Bladder pressure rose up to the 7th postoperative day and at day 21 was at a slightly lower level than at day 3. The changes were significant ($P < 0.05$) at all measurement points.

Bladder capacity

The cystometric capacities of test animals after laser treatment were expressed as a percentage of the ratio to the measurements of the same animals prior to PDT (Fig. 2). As bladder volume, the volume was counted when, in post-treatment measurement, the intravesical pressure exceeded the preoperative end filling pressure in each animal group. At day 1, the capacity was reduced in every group, but after that, recovery started in animals treated with ALA and 20 J/cm^2 or 40 J/cm^2 light dose. In the groups of ALA and 80 J/cm^2 light or 20 J/cm^2 light only, the bladder capacity was still reduced at 3 days and recovery had only started at day 7.

In the 20 J/cm^2 light group, the animals did not regain more than 34% of their capacity of control values at 21 days, whereas 50% of the capacity was regained in the group of 80 J/cm^2 light with ALA. Generally, more complete recovery was seen with lower doses of light and ALA. Even 100% and 97% of control capacity values was regained in the animals treated with ALA and 20 J/cm^2 or 40 J/cm^2 light.

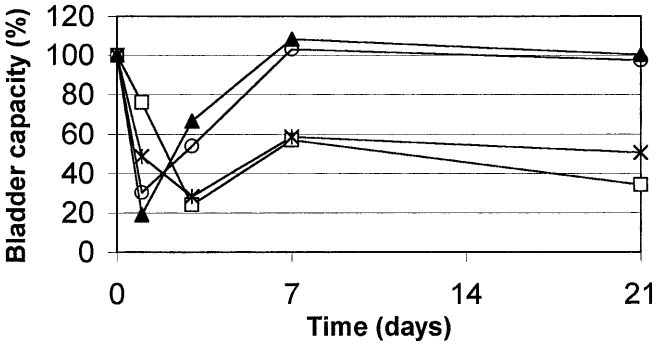

Fig. 2 Changes in rat bladder capacity after PDT using intravenous ALA photosensitizer (300 mg/kg) and 20 J/cm^2 (▲), 40 J/cm^2 (○), and 80 J/cm^2 (×) energy doses. Control group received 20 J/cm^2 (□) energy without ALA predisposition. Bladder capacity is expressed as a percentage of that in the same animals prior to PDT

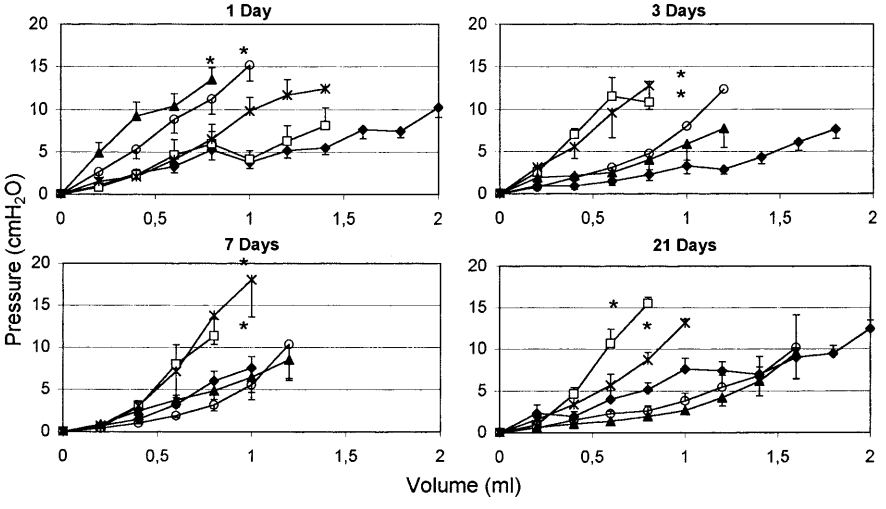

Fig. 1 Filling cystometry curves in rat bladder after PDT. Mean ± SEM in the same animals prior to PDT (◆) and 1, 3, 7, and 21 days after PDT using intravenous ALA photosensitizer (300 mg/kg) and 20 J/cm^2 (▲), 40 J/cm^2 (○), and 80 J/cm^2 (×) energy doses. Control group received 20 J/cm^2 (□) energy without ALA predisposition. *Asterisks* indicate statistical significance ($P < 0.05$)

Discussion

The most serious potential complication of whole-bladder photodynamic therapy is permanent bladder contracture, which has been reported in 13–40% of the patients treated [15,18]. Possibly those authors who have not commented on reduced bladder capacity after whole-bladder PDT have not specifically looked for it. Reduced bladder capacity may also be partly concealed by significant reflux and hydronephrosis as discovered on cystography in four out of five of patients with severe irritative symptoms [6].

Photodynamic therapy is primarily suggested for the therapy of papillary transitional cell carcinoma (TCC) and refractory carcinoma in situ (CIS), and prophylaxis of recurrent superficial TCC in those patients who have failed intravesical chemotherapy or immunotherapy [15]. It appears that photodynamic therapy can enable 31% of patients whose only remaining option is radical cystectomy to retain their bladders for up to 1 year without undue risk of disease progression. It is possible that some of these heavily pretreated bladders with severe fibrosis had coexistent vesicouretral reflux prior to PDT. Therefore, it is essential to avoid delivering excessive light-energy intensity during whole-bladder photoillumination in this patient group [18]. It is evident that by using treatment parameters that do not damage the bladder muscle, the resulting functional impairment is less severe and the bladder wall recovers more completely than by using treatments that do damage the muscle [20]. To find out these correct treatment parameters, the effect of PDT on normal bladder tissue becomes important. The goal for PDT therapy is to leave the muscular layer intact in order to avoid irreversible damage to the bladder wall and its functional impairment. The aim of this study was to determine the optimum amount of laser light needed to induce a reversible damage to the bladder wall and its functional capacity.

According to the results of this study, a light dose of 20 J/cm^2 without ALA induced 66% reduction in bladder capacity at 3 weeks, but 20 J/cm^2 with ALA (300 mg/kg) produced only transitory irritative bladder symptoms. Aminolevulinic acid-induced PpIX appears to localize preferentially in the mucosa rather than the muscle of hollow organs [2, 3, 5, 10, 11, 13, 22]. It would be expected that if the tissue concentration of the photosensitizer was below the threshold in the muscle but above it in the mucosa, then there might be a mechanism for limiting PDT effects to the superficial layers [19]. Possibly the relative selective retention of the photosensitizer in the mucosa protects the other layers of the bladder. Therefore, the functional changes remain minimal after reasonable light doses up to 40 J/cm^2, according to this study. Barr and co-workers found that the bursting strength of the colon following PDT is not reduced, despite full-thickness damage to the colonic wall. However, after thermal damage, immediate colon perforations were seen in 20%. The reduced bursting strength lasts for 2 weeks. Selective collagen strain studies indicated that the submucosal colonic collagen may not be damaged by PDT with A1SPc (aluminium sulfonated phthalocyanine), but the architecture is severely disrupted by thermal injury [1]. Our results also suggest that the photosensitizer must have a protective effect on the muscle layers and the functional capacity of the bladder.

The wide range of light doses used in clinical series of PDT for superficial bladder cancer is notable [4, 14, 16, 18, 19]. Our results revealed that light doses of 20 J/cm^2 [14, 15] and 40 J/cm^2 with ALA induced only irritative changes that subsided after day 1, but no functional changes. Pope and Bown also discovered that even quite low doses of PDT still produced a marked initial reduction in bladder capacity due to the acute inflammation, but these improved to within normal limits after about 1 month [20]. In that study, the dose of AlSPc varied together with the constant 20 J/cm^2 light dose. The control group received 20 J/cm^2 light alone and showed no significant functional changes after laser irradiation, which is contrary to our finding. Only with the highest doses of AlSPc were the functional changes in bladder compliance and capacity significant, and the animals treated did not regain more than half of their bladder capacity at 3 months. However, there was no long-term histologic abnormality apparent in animals after the acute inflammatory process had settled, even when there was still some functional impairment.

When the light dose was increased to 80 J/cm^2 with administration of ALA(300 mg/kg), there was a marked reduction seen in bladder capacity in this study. Animals treated in this group did not regain more than half of their bladder capacity at 3 weeks. In addition, four animals died or had major necrosis of the bladder in this therapy group. Both the light energy and photosensitizing chemical dosages are limiting factors for the final effect of PDT. The dosage of light is more difficult to control than that of chemicals, but this study shows that it is also possible to adjust it. Our results are in good correlation with the other known studies.

Clinically, with light doses of 60 J/cm^2 and 30 J/cm^2, Kriegmair and co-workers achieved a partial control of superficial bladder cancers with intravesical administration of ALA [9]. Nseyo and co-workers found that PDT using 1.5 mg/kg of Photofrin (hematoporphyrin derivative compound) and 15 J/cm^2 of light (630 nm), should be considered a safe and effective treatment for refractory CIS or recurrent papillary transitional cell carcinoma (TCC) [15, 16].

Conclusions

We conclude that the optimum light dose with ALA 300 mg/kg administration is 40 J/cm^2 for maximum and safe PDT effects in the rat bladder. There is only a transient disturbance of bladder function as long as a

relevant dose of light is used and muscle layer damage is avoided. When the light dose is enhanced to 80 J/cm^2, it is impossible to avoid such major complications as bladder permanent shrinkage with whole-bladder PDT. As there is only limited background data available on the functional changes related to PDT, it is also necessary to carry out these basic experiments.

Acknowledgements The authors wish to thank Jarmo Hallikainen, PhD, and Markku Tiirikainen, PhD, of the Department of Applied Physics in Kuopio University, for supplying the laser facilities, and Pharmacia & Upjohn for partial financial support for the current study.

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