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KINETICS, BIODISTRIBUTION AND THERAPEUTIC EFFICACY OF HEXYLESTER 5-AMINOLEVULINATE INDUCED PHOTODYNAMIC THERAPY IN AN ORTHOTOPIC RAT BLADDER TUMOR MODEL

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ABSTRACT

Purpose: To optimize photodynamic therapy (PDT) we investigated the kinetics and biodistribution of hexylester 5-aminolevulinate (hALA) induced protoporphyrin IX (PpIX) and the therapeutic efficacy of PDT at different drug and light doses in an orthotopic rat bladder tumor model.

Materials and Methods: Healthy and tumor bearing rats were instilled intravesically with hALA (4, 8 and 16 mM) for 1 hour. Fluorescence was recorded spectroscopically in situ. PpIX fluorescence distribution and quantification across the bladders was visualized with fluorescence microscopy. PDT efficacy at different fluences (15 to 80 J/cm^2) was histologically assessed 48 hours and 1 week after treatment.

Results: Spectroscopic analysis in normal or tumor bearing rats showed the highest tumor-to-normal ratios 2 or 3 hours after the end of the 8 or 16 mM hALA instillation (5.4 and 5.7, respectively). Within the same tumor bearing animal the same fluorescence levels were observed in normal epithelium and transitional cell carcinoma, whereas the tumor-to-muscle ratio was 3. Tumor necrosis with an intact normal bladder wall was observed with a fluence of 20 J/cm^2 for 8 mM hALA, while 15 J/cm^2 was ineffective and 25 J/cm^2 induced total wall necrosis. Although it induced comparable PpIX fluorescence, 16 mM hALA did not result in tumor eradication at any fluence.

Conclusions: An optimal PDT effect was obtained with 8 mM hALA and a fluence of 20 J/cm^2. While different hALA concentrations induce identical PpIX fluorescence intensities, the PDT outcome was considerably different. Thus, fluorescence does not necessarily predict the therapeutic efficacy of PDT.

KEY WORDS: bladder, bladder neoplasms, photochemotherapy, aminolevulinic acid, fluorescence

Since the first report of photodynamic treatment of bladder cancer by Kelly and Snell,[1] a considerable amount of research on this subject has been performed. Nevertheless, despite interesting clinical results in patients with carcinoma in situ (CIS) refractory to intravesical chemotherapy or immunotherapy photodynamic therapy (PDT) remains experimental and reports are limited to small series. The 2 major drawbacks of this technique (prolonged cutaneous photosensitization and detrusor fibrosis with consequent loss of bladder capacity) can be attributed to the administration route (intravenous) and poor selectivity of photosensitizers, namely hematoporphyrin derivatives, dihematoporphyrin esters and porphymer sodium (Photofrin II, Axcan Scandipharm, Birmingham, Alabama).

Leveckis et al first described the potential use of 5-aminolevulinic acid (ALA) in urology.[2] ALA is a precursor in the heme biosynthetic pathway that induces intracellular accumulation of endogenous protoporphyrin IX (PpIX) if provided exogenously in excess. Intravesical administration of ALA, resulting in epithelial PpIX accumulation, and blue light endoscopic examination to induce and visualize red fluorescence, is commonly used today in urological practice. Fluorescence guided cystoscopy increases the detection rate of bladder cancer by 20% and decreases the recurrence rate by 60%.[3, 4]

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Several studies have shown that the modification of a drug to an ester improves penetration through biological barriers in vitro and in vivo. Hexyl-ALA (hALA) appears to be ideally suited for urological applications since it offers a good compromise between water-urine solubility and lipophilicity.[5] In humans intravesical instillation of 8 mM hALA for 2 hours induces the same fluorescence intensity as 6 hours of 180 mM ALA.[6]

To our knowledge no studies today have investigated the effectiveness of hALA mediated PDT for bladder cancer in vivo. In the current study we investigated the kinetics and biodistribution of hALA induced PpIX and the therapeutic efficacy of PDT at different drug and light doses in an orthotopic rat bladder tumor model.

MATERIALS AND METHODS

Cell culture and tumor model. AY-27 cells from FANFT (N-(4-[5-nitro-2-furyl]-2-thiazolyl) formamide) induced rat bladder transitional cell carcinoma (TCC) were cultured in vitro as a monolayer. Xiao et al have previously described the orthotopic tumor model used.[7] Briefly, after anesthesia attained by intraperitoneal injection of 45 mg/kg sodium pentobarbital the bladders were catheterized with an 18 gauge cannula. Chemical desquamation was achieved by a 15′′ rinse with HCl 0.1N by 15′′ KOH 0.1N. An AY-27 cell suspension (10^6 cells in 0.5 ml medium) was then instilled into the bladder and maintained for 1 hour. Spectroscopy studies and PDT experiments were performed 3 and 1 weeks, respectively, after inoculation.

hALA preparation and intravesical administration. Concentrations of 4, 8 and 16 mM hALA were prepared in sterile phosphate buffered saline (pH 6). After catheterization 0.5 ml hALA solution was instilled in the bladder for 1 hour before measurements.

*In situ spectroscopy.*A total of 12 healthy and 12 tumor bearing rats were instilled with the different concentrations for 1 hour. In situ fluorescence spectroscopy was performed, as described previously.[8] Autofluorescence spectra were obtained prior to hALA administration. Fluorescence was measured in situ from the end of instillation at hourly intervals for 4 hours. Spectra were recorded at 3 spots in the bladder. To estimate PpIX fluorescence autofluorescence between 615 and 715 nm was substracted from the recorded signal in the same band width and normalized according to autofluorescence between 500 and 600 nm.

Fluorescence microscopy. Cystectomy was performed 2 or 3 hours after the end of instillation with 8 or 16 mM, respectively. Bladders samples were embedded in tissue freezing medium and kept in the dark at –20C for 1 hour, followed by the preparation of 6 μm frozen sections. Fluorescence microscopy was done as previously described.[8] Image J software (National Institute of Health, Bethesda, Maryland) was used to quantify fluorescence. Fluorescence measurements from normal epithelium, TCC and muscle were obtained from all samples and averaged for the different instillation conditions.

Whole bladder PDT. For PDT experiments each fluence group (15, 20, 25, 40 and 80 J/cm^2) consisted of 3 healthy and 5 tumor bearing rats as well as 2 healthy and 2 tumor bearing rats in the control group (light but no drug). Whole bladder irradiation was performed at 630 nm and provided by an argon pumped dye laser (Spectra Physics 2020, Les Ulis, France). The light was coupled into an optical fiber (400 μm) with a spherical light diffuser (Medlight S. A., Ecublens, Switzerland). The fiber was inserted into the bladder filled with 0.5 ml normal saline and fixed in a central position. The fluence rate was set at 100 mW/cm^2. PDT was performed 2 or 3 hours after the end of instillation with 8 or 16 mM hALA, respectively.

RESULTS

In situ fluorescence kinetics in normal and tumor bearing bladders. In normal bladders maximal fluorescence was achieved at the end of instillation with 4 mM solution with a plateau until 2 hours (mean ± SEM 4.5 ± 2.0 ru), after which it progressively returned to pre-instillation levels (fig. 1). Almost the same maximal fluorescence level was achieved 2 hours after 8 mM instillation (2.8 ± 5.0 ru), whereas 16 mM hALA did not induce significant PpIX fluorescence in normal bladders (1.8 ± 3.0 ru). A 4 mM hALA instillation in tumor bladders resulted in the same fluorescence levels as in normal bladders (6.5 ± 4.0 ru) with a maximal tumor-to-normal ratio of 1.4. The maximal fluorescence signal in tumor bearing bladders after 8 or 16 mM sensitization was achieved at 2 or 3 hours (15.5 ± 10.0 or 10.2 ± 9.0 ru, respectively) with a rapid decrease to pre-instillation levels at 4 hours. The maximal tumor-to-normal ratio of 5.4 and 5.7 was obtained 2 or 3 hours after the end of instillation with 8 or 16 mM hALA solution, respectively. Because of the poor tumor-to-normal ratio with 4 mM hALA, this concentration was abandoned in further experiments.

Fluorescence microscopy. Fluorescence microscopy showed that PpIX fluorescence appeared to be limited to normal or transformed epithelium at each concentration (fig. 2). However, quantification of fluorescence images revealed the presence of PpIX in the muscularis (fig. 3). In healthy bladders the epithelium-to-muscle ratio was 3 at 8 mM and 5 at 16 mM. TCC had slightly higher fluorescence intensity than adjacent normal epithelium at each concentration (1.2/1). A 16 mM instillation produced a normal epithelium-to-muscle ratio of 2.4 and a TCC-to-muscle ratio of 3. After 8 mM sensitization the corresponding ratios were 3.4 and 3, respectively.

PDT in normal and tumor bearing bladders. Light only at a fluence rate of 100 mW/cm^2 in healthy or tumor bearing rats did not alter histological findings except for a slight inflammatory reaction at 80 J/cm^2.

At 8 mM hALA histological examination of healthy bladders 48 hours after PDT did not demonstrate any alterations at 15 J/cm,^{2} whereas total epithelial denudation and focal wall necrosis were observed at 20 J/cm^2 (1 of 3 rats) or total wall necrosis from 25 up to 80 J/cm^2 (9 of 9). Fluences above 15 J/cm^2 in healthy rats resulted in animal death within 1 week (8 of 8 rats). Necropsy showed peritonitis and bladder perforation. Pathological findings in tumor bearing rats 48 hours after irradiation with 15 J/cm^2 revealed persistent TCC with an unaltered bladder wall (4 of 4 rats) (fig. 4, *A*). A fluence of 20 J/cm^2 resulted in complete tumor eradication with an intact normal epithelium and bladder wall (5 of 5 rats) (fig. 4, *B*). However, necropsy 1 week after PDT showed

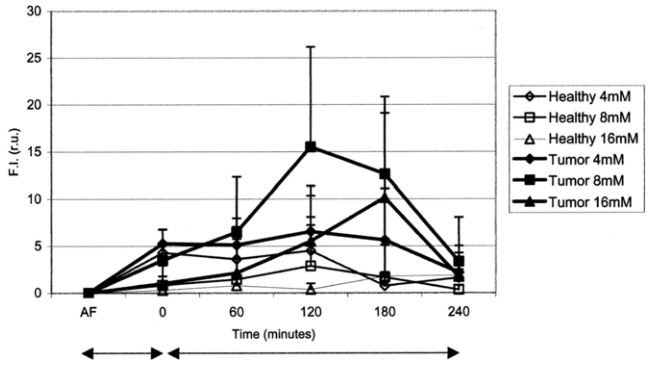

FIG. 1. In situ fluorescence kinetics in healthy and tumor rat bladders before and after 1-hour intravesical instillation of hALA at 4, 8 and 16 mM. Autofluorescence (*AF*) was measured before hALA instillation and PpIX fluorescence was measured at hourly intervals for 4 hours. *F.I.*, fluorescence intensity.

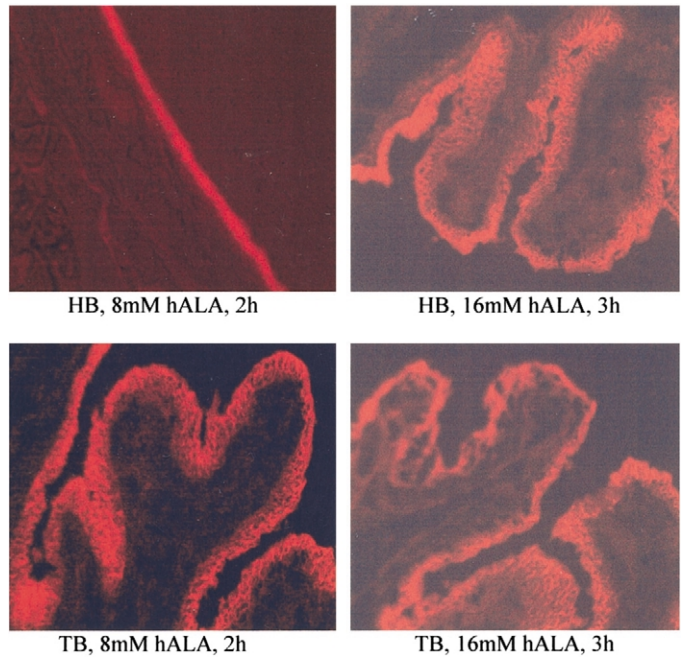

FIG. 2. Fluorescence microscopy of PpIX in healthy (*HB*) and tumor (*TB*) bladders 2 or 3 hours after end of intravesical instillation of 8 or 16 mM hALA. Reduced from ×100.