Photodynamic Selectivity of 5-Aminolevulinic Acid to Prostate Cancer Cells

SULTAN M. SULTAN, M.D.; ABDEL-ALIM M. EL-DORAY, M.D.; ALFONS HOFSTETTER, M.D. *; OSAMA ABDEL-GAWAD, M.D.; ALAA EL-DEEN EL-MAHDY, M.D. and WAEL KHODER, M.Sc.
The Departments of Urology, Minoufiya University, Egypt and Ludwig Maximilians University Hospital, Germany.*

ABSTRACT

Objective: To determine the selectivity of 5 – aminolevulinic acid (5-ALA) as a photosensitizer to malignant prostatic cells in men undergoing radical retropubic prostatectomy.

Patients and Methods: Nineteen patients with localized prostate cancer were included in the study. Eighteen patients received 5-ALA and one patient did not receive it and was used as a control. The dose was 20mg/kg body weight. 15 patients received 5-ALA 4 hours before radical prostatectomy, two patients received it 2 hours before prostatectomy through a Ryle tube, and one patient received 5-ALA 12 hours before the operation. The removed prostates were examined for protoporphyrin IX (PpIX) fluorescence macroscopically, by fluorescence microscopy and by light microscopy.

Results: All carcinomas showed a clear evidence of PpIX-enrichment except in the control case. The enrichments were strong (++ in 15 cases and weak (+) in 3 cases. Two of those three cases were given 5-ALA two hours through a Ryle tube before excision of the prostate as well as the patient who was given 5-ALA 12 hours preoperatively. No PpIX enrichment was observed in the stroma of the prostate gland or in the benign tissue sections in any case (0/19).

Conclusion: Oral 5-ALA is selectively concentrated in malignant cells of the prostate. This may lead to the clinical application of photodynamic therapy for localized prostate cancer.

Key Words: Prostate cancer – 5-aminolevulinic acid (5-ALA) – Photodynamic – Fluorescence – Protoporphyrin IX.

INTRODUCTION

Prostate cancer is the second leading cause of male death after lung cancer in USA. An effective therapy for adenocarcinoma of the prostate continues to elude scientific and clinical investigators due to its tumour biological viability, potential lethality and the unpredictability of its clinical course [1].

Developments in the field of photodynamic diagnosis and treatment have been in progress since the beginning of the 1980s. Photodynamic diagnosis system with core components of a laser, a special endoscopy system and a tumor marker produced very good results for early recognition of bladder tumors [2,3]. Photodynamic treatment (PDT) of the benign prostate has been evaluated with the use of various photosensitizers. Dihematoporphyrin ester (Photophrin) has been shown to cause glandular necrosis in the canine prostate [4]. However, there are several limitations of photophrin-mediated PDT in the prostate gland because of its limited depth of penetration and its association with prolonged (6-8 weeks) skin photosensitivity [5]. Hsi et al., [6] evaluated another photosensitizer called motexafin lutetium (LuTex) for treatment of canine prostate. They postulated good results with shorter skin sensitivity.

Protoporphyrin IX (PpIX) is produced in cells via the heme synthesis pathway, from the substrate aminolevulinic acid (ALA), and can be used for tumor detection, monitoring of the tumor or photodynamic therapy. Rapidly proliferating malignant cells may produce more ALA-
derived porphyrins. This provides a biological rationale for the clinical use of ALA-based PDT [7].

In the present study, the aim was to evaluate the selectivity of 5-ALA to prostate cancer cells in men undergoing radical retropubic prostatectomy.

**PATIENTS AND METHODS**

This study was conducted in Ludwig Maximilians University Hospital in Munich, Germany during the period from July 2002 to December 2004. Nineteen patients with increased serum PSA levels suspected to have organ-confined prostate cancer and who accepted to participate in the study were included. The diagnosis of organ confined prostate cancer was confirmed by TRUS biopsy, CT scan and MRI.

The age of patients ranged from 52 to 72 years with an average age at time of interference of 62.25±9 years. In eighteen patients, the histological diagnosis was carried out preoperatively by means of TRUS guided twelve zone biopsy scheme. In the remaining patient, carcinoma was accidentally discovered in TURP biopsy specimen.

5-Aminolevulinic Acid (5-ALA) was provided by Fa. Medac Gmbh, Theaterstr. 6, 22880, Wedel, Germany, in the form of dry powder (1.5gm bottles) to be dissolved in 50mL drinking water. 5-ALA was given orally to the patient immediately after dissolution in a dose of 20mg/Kg body weight.

According to the animal experimental kinetic studies [8] we have chosen the following scheme for, fresh 5-ALA ingestion. 15 patients received the 5-ALA 4 hours before the planned time of radical prostatectomy. Two patients received 5-ALA 2 hours before organ removal through a Ryle tube intraoperatively. One patient received 5-ALA 12 hours before the operation. One patient did not receive 5-ALA and was used as a control case to the study. Sections examined from stroma and benign prostate tissues were used also as controls for malignant cells regarding the existence of PpIX-Fluorescence.

After surgical excision of the prostate, the organ was immediately placed in a dark room and examined macroscopically for the existence of PpIX-fluorescence. The prostate was subsequently divided into longitudinal sections 0.5cm in thickness and the cuts were also examined for the existence of PpIX-fluorescence. The sections were then frozen to –70°C in liquid nitrogen and brought light-protected on dry ice for further histological examination.

The PpIX-fluorescence is produced by examination with blue light of the wavelength area λ=380-420nm. This is generated by means of the incoherent xenon light source (D-Light, Karl STORZ GmbH, Tuttlingen, Germany). The light is transported by means of a particular zero cystoscope lens with a yellow filter integrated in its ocular light fiber bundle. This yellow filter builds a colour contrast between the red PpIX-fluorescence and that of the re-scattered blue stimulation light represented from the surrounding normal tissue in which PpIX is not concentrated.

The frozen sections were examined by fluorescence microscopy and the corresponding cuts were stained with Haematoxylin & Eosin dyes and then examined for the identification of tumour localization and Gleason score by light microscope.

PpIX-enrichment was evaluated in stroma, benign tissues and carcinoma and classified by the examiner as strong (++), weak fluorescence (+), or no fluorescence (–).

The patients were protected from direct sunlight or strong light for the first 48 hours after 5-ALA ingestion. Liver and kidney function tests were done postoperatively to be correlated with the preoperative baseline values. Routine observations of vital signs pre, intra and postoperatively were regularly done.

**Statistical methods:**

Data were expressed as mean ± SD. Chi-square test was used to determine the association between the PpIX fluorescence and the Gleason score. p value <0.05 considered significant. The sensitivity and specificity of the selectivity of 5-ALA to malignant cells were also calculated.

**RESULTS**

Adenocarcinoma was found in all histopathological specimens. Gleason score is shown in Table (1).
In macroscopic fluorescence examination, all malignant prostates showed a clear evidence of PpIX-fluorescence except the prostate of the control patient. In the fluorescence microscopic examination the enrichments (fluorescence intensity) were strong (++) in 15 cases and weak (+) in 3 cases. From these three patients, two were given 5-ALA two hours through a Ryle tube before prostatectomy and one patient was given 5-ALA 12 hours preoperatively.

No PpIX fluorescence was observed in the stroma of the prostate gland or in the benign tissues of the prostate in any case (0/19) (Illustrations 1, 2).

The sensitivity and specificity of 5-ALA to malignant cells were 73% and 100%, respectively, as shown in Table (2).

No significant association was detected between the PpIX-enrichment of the carcinomas in reference to the Gleason Score ($p>0.05$). (Table 3).

There were no pre or post-operative complications in all patients. These included cutaneous phototoxicity, cardiopulmonary disturbances or gastrointestinal complications. The postoperative liver and kidney functions were not changed as compared with the preoperative values in all patients.

Illustration (1): PpIX-Enrichment in a prostate carcinoma (Gleason 6). Light microscopic sections (Low power magnification of H&E stain) (Left) and corresponding Fluorescence microscopy (Right). The PpIX-enrichment is located only in the carcinoma areas. No PpIX is found in the neighbouring stroma.

Illustration (2): PpIX-Enrichment in a prostate carcinoma (Gleason 6) High power field section (H&E stain) (Left) and corresponding frozen section in fluorescence microscope (Right). This magnification shows that the PpIX-enrichment absolutely occurs in carcinoma areas (200X).
DISCUSSION

Although several sensitizers other than 5-Aminolevulinic Acid (5-ALA)-induced Protoporphyrin IX (PpIX) have been used in clinical photodynamic therapy (PDT), ALA-PDT was the most active field in PDT research. The number of published articles in both basic and clinical research on ALA-PDT has been increasing exponentially [9,10].

In biological systems, PpIX is an intermediate in the biosynthesis of the Fe$^{2+}$ containing haeme. Haeme biosynthesis is an essential pathway in cell metabolism. Due to the excessive cell proliferation and division and the slow conversion into haeme from PpIX, malignant cells and tissues both accumulate substantially more PpIX than normal cells and tissues [11].

5-aminolevulenic acid is used either topical or systemic for the photo-dynamic therapy. The topical application is used mainly in dermatology for the treatment of acute keratosis and psoriasis [12,13].

In urology, PDT with 5-ALA was first introduced after successful systemic sensitizer application. Intra-vesical 5-ALA application was used successfully for diagnosis [2,14] as well as photo-dynamic therapy [15,16] of superficial bladder cancers.

Systemic administration of 5-ALA is currently used also for the treatment of malignant brain tumors [17], in gastroenterology for esophageal and bile-duct carcinomas [18] and in gynaecology as vulvar and cervical carcinoma [19]. So, we planned to examine 5-ALA selectivity to prostate malignant cells in this study.

Higher doses of 5-ALA would induce greater fluorescence, but undoubtedly, it may induce more side effects [7]. The dosage of 5-ALA used orally in our study has been reported to induce minimal side effects. After oral administration of 20mg/kg of 5-ALA, there was no change in laboratory-tested variables and no photophobia or vomiting were noticed in our patients.

The experimental animal examinations on the PDT with 5-ALA induced PpIX for renal cell carcinoma [20] as well as bladder cancer [21] had shown an interval of approximately 4-6 hours to the maximum PpIX intensity after oral 5-ALA. For this reason, oral 5-ALA was given to 15 patients 4 hours before the expected removal of the prostate, 2 hours and 12 hours preoperatively in 2 patients and one patient, respectively.

In the present study, the maximum tissue concentration of PpIX occurred at 4 hours after the oral administration of 5-ALA. The patients who got the application of 5-ALA through the Ryle tube during the intervention and consequently had a PpIX incubation time of maximum 1.5-2 hours showed weaker fluorescence intensity. The weaker fluorescence intensity after 1.5-2 hours of incubation period was confirmed also by the animal experimental PpIX kinetics that also proved weaker fluorescence intensities in this time period [22]. The patient to whom 5-ALA was administered 12 hours before surgery showed weaker fluorescence intensity also.

In our study, the selective enrichment of PpIX in the human prostate cancer cells was proved. In the fluorescence microscopic examination, it was shown that all examined prostate carcinomas showed PpIX fluorescence irrespective of their degree of differentiation.
In contrast to the examinations of the benign beagle prostate [23] there was no PpIX accumulation in the human benign glandular or stroma cells. Consequently a sufficient sensitivity in the human carcinoma for the 5-ALA induced PpIX accumulation 4-6 hours after application can be postulated as an ideal character for its photodynamic effect.

**Conclusion:**

PpIX fluorescence is selectively concentrated in malignant cells of the prostate after oral administration of 5-ALA. Oral 5-ALA as a photosensitizer to malignant prostatic cells is a feasible method. This may lead to further clinical trials of photodynamic therapy as a new modality for treatment of localized prostate cancer.

**REFERENCES**


