IN-VIVO AND IN-VITRO PHOTODYNAMIC DIAGNOSIS OF BLADDER TUMORS

PhD Thesis by Ákos Pytel M.D.

Program leader: Árpád Bellyei MD., PhD.,Med.Habil.

University of Pécs, Medical School

Department of Urology

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AIM OF THE STUDY

Review the literature regarding fluorescence cystoscopy, and comparison of the different fluorophores.

Examination of different photosensitivation during fluorescence cystoscopy, and comparison of the data regarding sensitivity and specificity.

Establish a new diagnostic method in the diagnosis and in the follow up of bladder tumours, which could reduce the number of cystoscopies.

Examination of different fluorochromes during fluorescence cytology, and comparison of the results regarding sensitivity.

IN-VIVO PHOTODYNAMIC DIAGNOSIS OF BLADDER CANCER

Review

5-ALA induced fluorescence cystoscopy

Kriegmair et al. were the first to describe intravesical ALA in humans. Since that 5-ALA induced fluorescence cystoscopy is widely used in urology. A total of 68 patients with bladder cancer were instilled with a 3% ALA solution for one to three hours, followed by blue light examination of the bladder. The fluorescence was observed through standard cystoscope and lenses. A sensitivity and specificity of 100% and 68.5%, respectively were obtained. In a larger series (106 patients), these results were further confirmed.

All reports on ALA-induced PpIX guided fluorescence detection confirm the high sensitivity to detect bladder cancer, including carcinoma in situ. The sensitivity and specificity however varie greatly from study to study; ranging from 83-100% (sensitivity) and from 42-84% (specificity). This is partly due to inexperience in blue light endoscopy.

Hungarian experiences were reported by Székely et al, and Sapanidis et al.

H-ALA induced fluorescence cystoscopy

Because the lipid bilayer of biological membranes is relatively impermeable to charged molecules, the cellular uptake of ALA is shallow. Systematic studies have shown that the modification of a drug to an ester improves penetration trhough biological barriers in vitro and in vivo. ALA esters have been shown to increase the intracellular build-up of PpIX, at drastically reduced concentrations and incubation times as compared to ALA in vitro Hexyl-ALA has been shown to be a good compromise between the water–urine solubility and lipophilicity.

Regarding the demonstrated data of Jichlinski et al. and the Hexvix PCB 301/01 Study Group, the sensitivity and specificity of H-ALA induced fluorescence cystoscopy is comparable of those with first generation protoporphyrin IX pecursores.

Hypericin induced fluorescence cystoscopy

Hypericin is a hydroxylated phenantroperylenequinone, present in a number of plants of the genus Hypericum, widely distributed around the world, the most common of which is St John’s wort. Hypericin is a pigment that produces singlet oxygen efficiently upon light irradiation with a quantum yield of 0.73. Being a potent photosensitizer hypericin is ideal for fluorescence cystoscopy.

D'Hallewin et al. investigated the possible use of hypericin as diagnostic tool for the fluorescence detection of bladder cancer. In a first series, 40 patients were instilled for at least 2 hours with 40 ml of an 8 μM hypericin solution. The diagnostic system and technique used for fluorescence detection was identical to the previously mentioned (D-Light System, Storz Company). All papillary areas showed bright red fluorescence. The sensitivity for detecting CIS was 93%. Seven out of ten non-fluorescent biopsies, containing carcinoma in situ, showed severe desquamation. The specificity was very high: 98.5%. In a larger series of 87 patients, the sensitivity and specificity for detecting flat carcinoma in situ were 94% and 93%, respectively.

Own experiences

Material and method

A total of 89 patients were investigated. The study was accomplished between June 2000 and December 2003 at the Department of Urology, University of Pécs and at the Department of Urology and Andrology St. Johannspital LKH Salzburg. The mean age of the patients was 65,2 year (range 41 to 84). 51 patients had a history of bladder tumors and 28 of them had previous intravesical therapy (BCG or mytomycin). The urine sediment analysis was negative in 29 cases, 45 patients had microscopic, 15 patients macroscopic hematuria.

With a 10 French catheter we instilled a solution of 1.5 g ALA in 50 ml 1.4% sodiumbicarbonat intravesicaly in 59 cases. In 18 cases 40 ml of a 8 micromolar solution of hypericin (0.16 mg hypericin, 4 micromolar polyvinilpyrrolidone 10 (PVP 10), 4 micromolar polyvinylpyrrolidone 40 (PVP 40) in 40 ml 0.9% NaCl solution) and in 12 cases 1g hexyl-aminolevulinic acid (Hexvix®) in 50 ml 1.4 % sodiumbicarbonat was instilled. The time of intravesical retention was between 60 minutes and 120 minutes.

We performed white-light, and fluorescence cystoscopy. All suspicious alterations were resected transurethraly. Altogether 342 biopsies were performed. We have used for fluorescence cystoscopy D-light® light source, 30, 70 degree endoscope and Urocam® PDD camera (Karl Storz Ltd.).

5-ALA induced fluorescence cystoscopy

205 biopsies in 59 patients were carried out. 112 samples were malignant, in 93 cases there were no evidence of malignancy. From the 112 positive samples 109 had induced fluorescence during fluorescence cystoscopy. Three Ta GI uroepithelial carcinomas were missed by PDD. From the 93 non malignant samples 14 had induced fluorescence. All of the fals positive cases received prior the intervention intravesical instillation therapy.

White-light cystoscopy missed 17 malignant diseases, and 79 white-light cystoscope guided biopsies were unnecessary.

In our series the sensitivity and specificity of 5-ALA induced fluorescence cystoscopy were 98% and 82% respectively.

H-ALA induced fluorescence cystoscopy

We have performed 61 biopsies in 12 patients. 39 samples were malignant, in 22 cases there were no evidence of malignancy. From the 39 positive samples 38 had induced fluorescence during fluorescence cystoscopy. One high grade dysplastic lesion was missed by PDD. From the 22 non malignant samples 6 had induced fluorescence. All of the fals positive cases received prior the intervention intravesical instillation therapy.

Conventional cystoscopy missed 8 malignant diseases, and 16 white-light cystoscope guided biopsies were unnecessary.

In our series the sensitivity and specificity of H-ALA induced fluorescence cystoscopy were 97% and 72% respectively.

Hypericin induced fluorescence cystoscopy

76 biopsies in 18 patients were performed. 33 samples were malignant, in 43 cases there were no evidence of malignancy. All positive samples had induced fluorescence during fluorescence cystoscopy. From the 43 negative samples 8 had induced fluorescence. All of the fals positive cases received prior the intervention intravesical instillation therapy.

White-light cystoscopy missed 8 malignant alterations.

The sensitivity and specificity of hypericin induced fluorescence cystoscopy were 97% and 72% respectively.

Summary of the datas

In our study 342 biopsies in 89 patients were carried out. 184 samples verified malignant urothelial lesions, in 158 cases there were no evidence of malignancy. Altogether 208 PDD guided biopsies were performed. Out of these areas were 145 suspicious under conventional cystoscopy as well. 133 white-light endoscopy guided biopsies were performed from non fluorescent areas. From the 184 positive samples 180 had induced fluorescence during fluorescence cystoscopy. Three Ta GI uroepithelial carcinomas and one high grade displasctic lesion were missed by PDD. From the 158 non malignant samples 29 had induced fluorescence. All of the fals positive cases received prior the intervention intravesical instillation therapy.

Conventional cystoscopy missed 33 malignant diseases (6 dysplastic lesions, 19 CIS, 4 TaGI, 1 T1GI, 1 T1GII, 2 T1GIII) and 129 white-light cystoscope guided biopsies were unnecessary.

In our series the overall sensitivity and specificity of induced fluorescence cystoscopy were 98% and 82% respectively.

Conclusions

Intravesical instillation of fluorophores, with limited systemic absorption and with limited systemic side effects, is the method of choice when considering fluorescence diagnosis of bladder cancer. Three fluorophores can be proposed to this purpose: ALA, h-ALA and hypericin. All three have been shown to have an excellent sensitivity (>90%), with various specificities ranging from 70 over 80 to 90%, respectively. Advantages from h-ALA

and hypericin over ALA consist in the low concentration required, and reduced photobleaching. Fluorescence-guided endoscopy has been shown to increase the amount of detected tumors by 30%, and resulting in a reduction of tumor recurrence.

Fluorescence cystoscopy is a safe method of excellent sensitivity. Beside exophytic lesions, „subendoscopic" flat malignant areas can be detected. Since the high sensitivity of the method, unnecesary random biopsies can be avoided.

After intravesical instillation therapy we must calculate with false positive cases.

IN-VITRO PHOTODYNAMIC DIAGNOSIS OF BLADDER CANCER

Material and method

A total of 78 patients were investigated in this study before undergoing transurethral resection (TURB). Only patients with a suspicion of bladder tumor diagnosed in out-patient cystoscopy were included in this pilot study.

The mean age of the patients was 66,7 year (range 47 to 84). 42 patients had a history of bladder tumors and 23 of them had previous intravesical therapy (BCG or mytomycin). The urine sediment analysis was negative in 25 cases, 39 patients had microscopic, 14 patients macroscopic hematuria.

With a 10 French catheter we instilled a solution of 1.5 g ALA in 50 ml 1.4% sodiumbicarbonat intravesicaly in 48 cases. Several months after starting our experiments with ALA we had the chance to obtain H-ALA and hypericin solution. Then we started to examine this fluorescent compounds also. In 18 cases 40 ml of a 8 micromolar solution of hypericin (0.16 mg hypericin, 4 micromolar polyvinilpyrrolidone 10 (PVP 10), 4 micromolar polyvinylpyrrolidone 40 (PVP 40) in 40 ml 0.9% NaCl solution) and in 12 cases 1 g hexil-aminolevulinic acid (Hexvix®) in 50 ml 1.4% sodium bicarbonat was instilled. The time of intravesical retention was between 60 minutes and 120 minutes. After incubation time of at least one hour, urine specimens were obtained for conventional and for fluorescence cytology. After that patients underwent TURB.

The induced fluorescence of urothelial cells were detected after centrifugation (1500 r/min for five minutes) and resuspension with a fluorescence microscope (Leica DM L). We used a band pass filter (BP 380-425 nm) for the excitation and a long-pass filter (LP 470 nm) for the emission. In case of detection of induced fluorescence of the urothelial cells the fluorescence cytology was defined as positive. In case of positivity we could detect red fluorescent cells beside the green appearing normal urothelial cells (Fig 1, 2). No pathologist was involved in the evaluation of the flourescence cytological probes. The results were compared with the histological findings and conventional cytology.

Results

Of the 78 patients in 70 cases urothelial carcinoma, in 8 cases non-specific inflammation was diagnosed by pathologists. 32 patients had Ta, 17 patients T1, 8 patients had carcinoma in situ and 13 patients invasive bladder cancer (T2-T4). The grading of the probes was Grade I in 30 patients, Grade II in 15 and Grade III in 25 patients. Five patients diagnosed with non-specific inflammation were instilled with ALA, and three with hypericin.

In the 70 patients diagnosed with malignant disease we could detect red fluorescent cells in 69 cases, while conventional cytology was positive in 53 cases. The overall sensitivity of fluorescence cytology was 98%, of the conventional cytology 76%.

5-ALA induced fluorescence cytology

Of the 48 patients who had an instillation with ALA we could detect red fluorescent cells in 44 cases. There were two false positive finding. In the 43 hystologicaly positive cases induced fluorescence could be detected in 42 cases, while conventional cytology was positive only in 33 cases. In this series the sensitivity of 5-ALA induced fluorescence cytology was 98%, while those of conventional cytology 77%.

Hypericin induced fluorescence cytology

After instillation of hypericin we could detect induced fluorescence in all hystologicaly positive cases. From the three negative patients in one we had false positive fluorescence cytology. Conventional cytology was in three cancer patients negative. In this subgroup of our study the sensitivity of fluorescence cytology was 100%, while the sensitivity of conventional cytology 80%.

Hexil-ALA induced fluorescence cytology

Of the 12 patients who had an instillation with H-ALA we could detect red fluorescent cells in all cases, and all of these patients had positive hystology as well. Conventional cytology was positive in 8 cases.

The sensitivity of H-ALA induced fluorescence cytology was 100%, and the sensitivity of conventional cytology in this group 67%.

Comment

The statistical analysis of the results shows a high sensitivity of the new method although the number of cases is still small. Flat urothelial neoplasias are difficult to visualize with conventional cystoscopy or with the less invasive flexible cystoscopy. Our preliminary data suggest good diagnostic value of the fluorescence cytology even in case of flat lesions. The detection of fluorescent cells is much easier than the detection of atypical cells in conventional cytology. Neither erythrocytes nor leucocytes have shown induced fluorescence and neither the grading nor the staging of the tumors had any influence for the diagnostic

value of the method. Intravesical BCG or chemotherapy in the history does not seem to influence the sensitivity and specificity of the test. The procedure was well tolerated in all cases, no side effect had been observed. The learning curve for the method of fluorescence cytology is minimal. The reduction of fluorescence during exposure to light is called photobleaching. Photobleaching causes a reduction in visible fluorescence during fluorescence endoscopy. This effect is induced both with white and with violet light, but it is much stronger with violet light. Using ALA and H-ALA for fluorescence cytology we could observe a strong photobleaching effect in our study. The red fluorescence of urothelial carcinoma cells fell below the detection limit in few seconds. By the use of hypericin we could not detect the photobleaching of the red fluorescent cells, which made the procedure easier for detection and documentation. The data published in the literature on the sensitivity and specificity of PDD for detection of bladder tumors with ALA and its derivate is comparable with our statistical data. Papers published recently on the use of hypericin for PDD have shown higher specificity with an excellent sensitivity. The selective cellular uptake of hypericin by urothelial carcinoma cells is demonstrated in the literature, so we are convinced that the specificity of our method is superior by using hypericin. Because of the possible higher specificity and because of the lack of the photobleaching phenomenon we recommend the use of hypericin for fluorescence cytology.

Conclusions

Fluorescence cytology is less invasive than cystoscopy and our preliminary results suggest that it is more sensitive than other noninvasive tests for the diagnosis of bladder cancer. The detection of induced fluorescence of malignant urothelial cells is easy and the learning curve is minimal, making the fluorescence cytology appropriate for daily practice. After further investigation it may become a new diagnostic method which may reduce the number of cystoscopies in the follow-up of bladder tumors.

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