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CLINICAL FLUORESCENCE DIAGNOSIS OF HUMAN BLADDER CARCINOMA FOLLOWING LOW-DOSE PHOTOFRIN INJECTION

L. Baert[1], R. Berg[2], B. van Damme[3], M.A. D'Hallewin[1], J. Johansson[2], K. Svanberg[4] and S. Svanberg[2]

[1]Dept. of Urology, St. Pieter University Hospital, University of Leuven, Brusselsestraat 69, B-3000 Leuven, Belgium

[2]Dept. of Physics, Lund Institute of Technology, P.O. Box 118, S-221 00 Lund, Sweden

[3]Dept. of Pathology, St. Rafael University Hospital, University of Leuven, Brusselsestraat 69, B-3000 Leuven, Belgium

[4]Dept. of Oncology, Lund University Hospital, S-221 85 Lund, Sweden

ABSTRACT

A point-monitoring fluorescence diagnostic system based on a low-energy pulsed laser, fiber transmission optics and an optical multichannel analyzer was used for diagnosis of patients with bladder malignancies. 24 patients with bladder carcinoma, carcinoma *in situ* and/or dysplasia were injected with 0.35 or 0.5 mg/kg body weight with Photofrin 48 hours prior to the investigation. The ratio between the red sensitizer emission and the bluish tissue autofluorescence proved to provide excellent demarcation between papillary tumours and normal bladder wall. Certain cases of dysplasia could also be differentiated from normal mucosa. Benign exofytic lesion such as malakoplakia appeared different from malignant tumours in fluorescence. Flat suspicious bladder mucosa such as seen in infectious diseases or after radiation therapy appeared normal in terms of fluorescence.

1. INTRODUCTION

Laser-induced fluorescence (LIF) is a relatively novel but attractive tool in tumour diagnostics.[1,2] In connection with fibre optics it has shown to be useful for localizing malignant tumours in the bronchial tree,[3] and in the urinary bladder[4-6] among several locations. LIF is of particular value in the diagnosis of early tumours, such as occult cancer not visible to the eye nor detectable with standard diagnostic methods. In order to obtain a significant fluorescence demarcation between malignant tumour and surrounding tissue, a fluorescent drug is injected about 48 hours prior to the investigation. A clinically used drug is Hematoporphyrin Derivative (HpD)[7-12] or its purified form Photofrin with a characteristic dual-peaked fluorescence structure in the red wavelength region. As

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the drug is retained in malignant tissue the fluorescence is characterized by the red dual peaked fluorescence in the tumour areas, which is not seen in normal tissue. An unwanted side-effect when using HpD is a skin photosensitization during a period of about 4-6 weeks after injection. As the skin effect is dose dependent the problem can be avoided by using low dose injection in conjunction with sensitive diagnostic equipment. During the last years a large variety of other fluorescent drugs have been developed such as phthalocyanines, chlorin and benzoporphyrin (BPD). One of the most promising is BPD[13] which does not give rise to a prolonged skin sensitization although with good tumour sensitizing properties. The intrinsic fluorescence from the tissue, the autofluorescence, can also be incorporated in the fluorescence characterization of biological tissue. It has been shown to decrease in malignant tumours compared to normal tissue for UV excitation.[14] In the demarcation of malignant tumours it is advantageous to use both the HpD related fluorescence and the autofluorescence to enhance the tumour demarcation. The drug specific fluorescence and the autofluorescence can also be used to form a ratio which has the advantage of being dimensionless. Such a ratio will be independent of the distance to and the topography of the object and also to laser intensity variations, parameters that might be difficult to control in clinical use. Furthermore, in many cases the shape of the autofluorescence might contain information that can be used to increase the demarcation between tumour and surrounding tissue.[14-17] This has been observed for many different tumour types using a nitrogen laser (337 nm) as an excitation source.

In this paper we report on an investigation of various types of bladder malignancies in 24 patients injected with 0.35 or 0.50 mg/kg b.w. of Photofrin. Bladder carcinoma and carcinoma *in situ* were easily demarcated from normal mucosa not only on the basis of the porphyrin fluorescence but also due to the different autofluorescence in the blue spectral region. Fluorescence spectra were also recorded from several cases of areas with dysplasia. The spectral shape was different for different grades of dysplastic transformation.

2. MATERIAL AND METHODS

2.1. Fluorescence recordings. As an excitation source a nitrogen laser (Laser Science VSL 337) was used alone or in conjunction with a dye laser, producing 3 ns pulses at 337 nm and 405 nm, respectively. The laser light was directed into a 600 μm fluorescence free quartz fibre, which was placed in contact with the object. The fluorescence light was collected by the same fibre and directed into an optical multichannel analyzer (EG&G PARC OMA III model 1460) equipped with an image-intensified 1024 element diode array detector. The overall spectral resolution of the system was about 10 nm. The system is described in detail in Ref. 17.

2.2. Urinary bladder tumours. The patients were intravenously injected with Photofrin, a purified form of HpD (QLT, Vancouver, Canada) 48 hours prior to the investigations. Two drug doses, 0.35 and 0.5 mg/kg b.w., were used. Fluorescence spectra from various kinds of superficial lesions in the bladder wall were recorded, such as different kinds of dysplasia, carcinoma *in situ*, superficial papillary bladder tumours and also benign lesions. The investigations were performed in connection with cystoscopy with the optical fiber through the biopsy channel of the endoscope. A biopsy specimen of each investigated area was taken for histopathological examination. Excitation at 337 nm or 405 nm were used.

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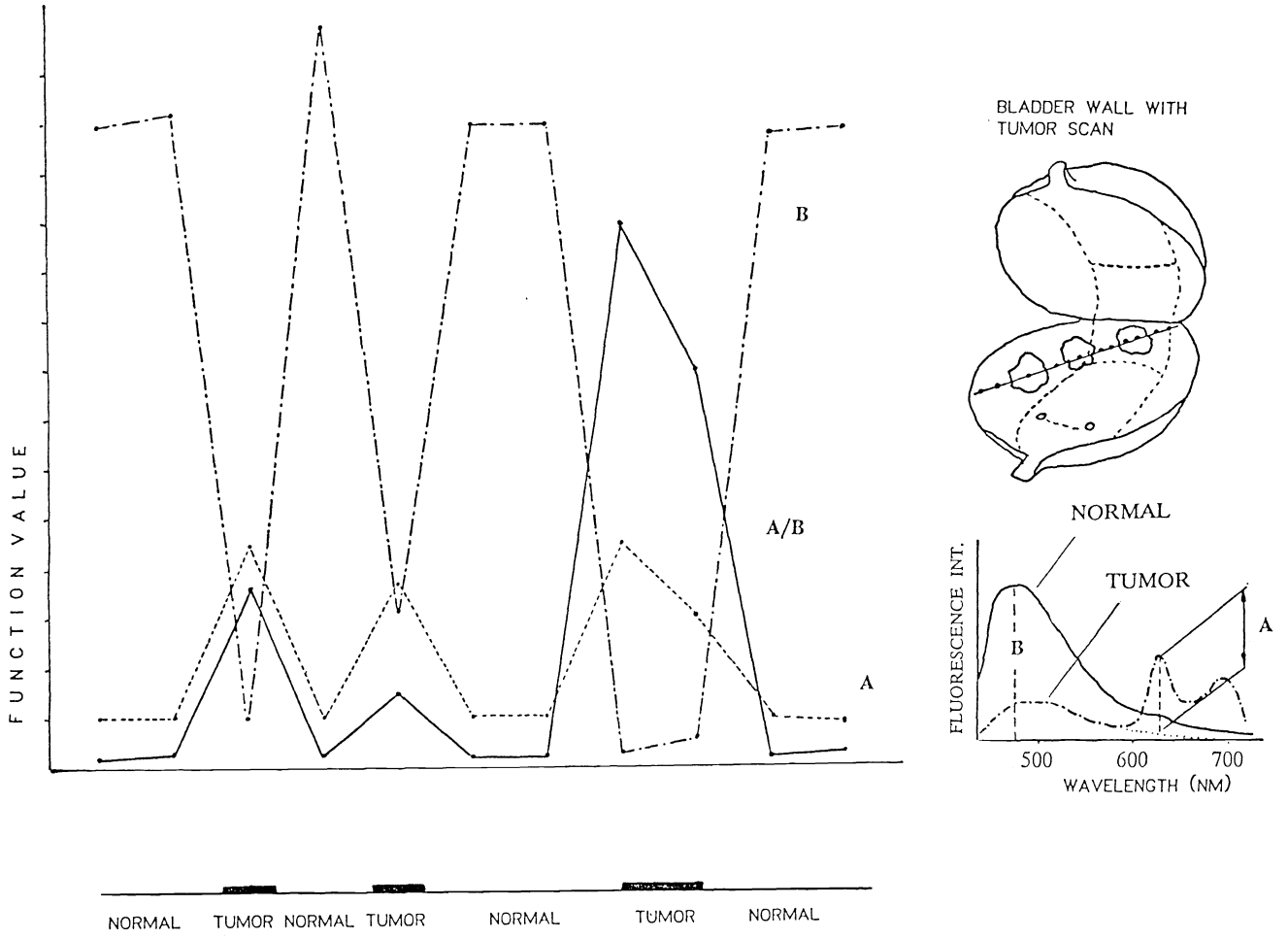

Fig. 1. Fluorescence data obtained in a scan across three malignant superficial bladder tumors with normal mucosa surrounding the lesions. The fluorescence investigation was performed during cystoscopy in connection with the diagnostic procedure. The fibre probe was placed through the biopsy channel of the endoscope. The patient had been injected with 0.35 mg/kg b.w. Photofrin 48 hours prior to the investigation. The location of the bladder tumors as well as two typical fluorescence spectra of tumor and normal mucosa are shown to the right. (From Ref. 18).

3. RESULTS AND DISCUSSION

The evaluated data from a scan across three flat malignant bladder tumours with normal surrounding mucosa in between are shown in Fig. 1. The patient was injected with 0.35 mg/kg b.w. Photofrin. The excitation wavelength was 405 nm. Two typical fluorescence spectra from tumour and normal mucosa are also shown. The autofluorescence signal at about 480 nm is denoted B, while the drug specific fluorescence at 630 nm is marked with an A. As seen in the figure there is a clear sign of the drug related dual-peaked fluorescence at about 630 nm and 690 nm in the tumour spectrum, while no such porphyrin signature can be recognized in the spectrum from the normal mucosa. Another clear difference between the two spectra is the intensity of the autofluorescence (B), with a lower intensity in the tumour

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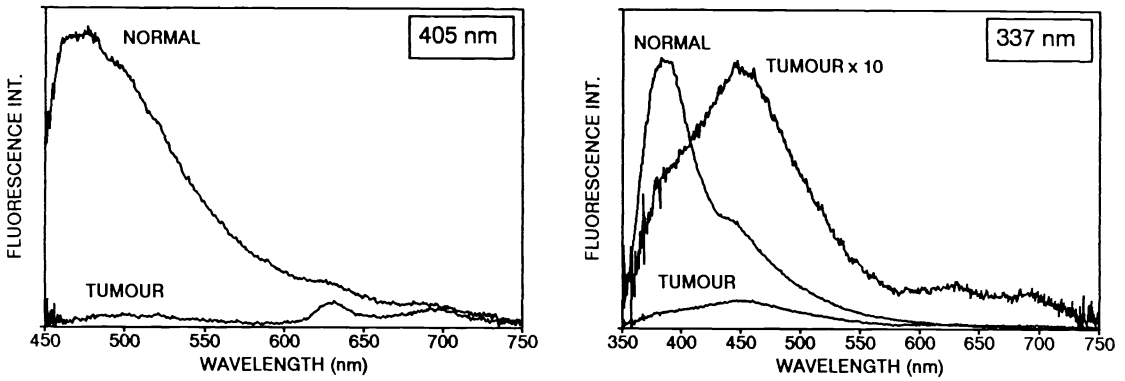

Fig. 2. Fluorescence spectra from a malignant superficial bladder tumour and normal mucosa. The excitation wavelength was 405 nm (left) and 337 nm (right). The tumour spectrum obtained at 337 nm excitation is also shown for 10 times intensity magnification. The patient had been injected with 0.35 mg/kg b.w. Photofrin 48 hours prior to the investigation.

area and a higher in the normal mucosa. If the drug specific fluorescence at 630 nm is divided by the autofluorescence signal at 480 nm an enhanced demarcation of the tumour area is obtained. This is illustrated in Fig. 1 where the intensities of A and B are plotted together with the dimensionless ratio A/B.

In Fig. 2 fluorescence emission spectra from normal bladder mucosa and a superficial malignant tumour are shown for excitation at wavelengths 405 nm and 337 nm in a patient injected with 0.35 mg/kg b.w. Photofrin 48 hours prior to the investigation. As can be seen in the figure the tumour area is recognized by a very low autofluorescence intensity in the blue-green wavelength region for both excitation wavelengths. The drug specific signal at about 630 nm can be identified for both excitation wavelengths although better at 405 nm, which is close to the prominent absorption peak for porphyrin at about 400 nm. The spectra excited at 337 nm show another difference in the autofluorescence with the maximum intensities occurring at wavelengths separated by 80 nm. The prominent fluorescence contribution at about 390 nm for the normal mucosa is not present for the malignant tumour.

In Fig. 3 we give some examples of the fluorescence signature of two different stages of dysplastic transformation, mild and severe dysplasia in the bladder wall together with the signature of normal mucosa. The autofluorescence peaking at about 480 nm shows a decreasing intensity in the regions of dysplasia. Beside the difference in the autofluorescence there is also a sign of porphyrin in the spectra from the dysplastic regions, which is not seen in the spectrum from the normal mucosa. It is interesting to note the successive transformation of the fluorescence spectra when going from normal mucosa to severe dysplasia, which are similar to the spectra obtained from carcinoma *in situ*. A known chromophore fluorescing at about 470 nm is NADH. It has been suggested by us and others that the decreased autofluorescence in malignant tissue might be due to less content of NADH.[18-21] The strong intrinsic fluorescence peaking at 390 nm recognized for 337 nm excitation in Fig. 2 might be due to the tissue content of collagen.[18]

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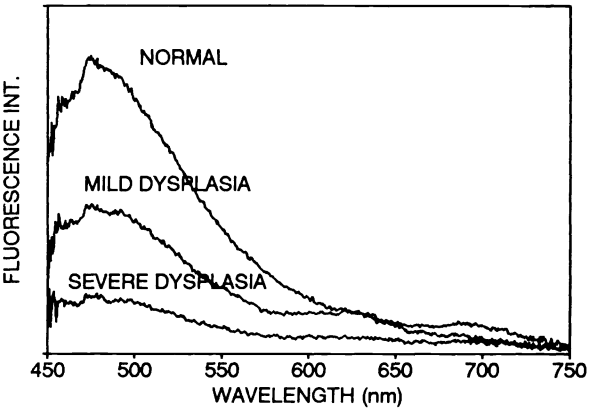

Fig. 3. Fluorescence spectra from normal urinary bladder wall and from different grades of dysplasia. The excitation wavelength was 405 nm. The patient had been injected with 0.35 mg/kg b.w. 48 hours prior to the investigation.

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