New Tool for Optical Biopsy Based on Endogenously Produced Protoporphyrin IX

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Introduction

Photodynamic therapy (PDT) and photodynamic diagnosis (PDD) for cancer patients have developed as important new treatment and diagnostic modalities in the past 25 years. One molecule potentially useful for both PDT and PDD is aminolevulinic acid (5-ALA), which is a precursor in the heme synthesis pathway and is widely used to induce fluorescent endogenous protoporphyrin IX (PPIX).

The traditional fluorimetric methods of detecting PPIX is by excitation at 405 nm and measuring red fluorescence emission at 633 nm (Shimadzu Spectrofluorophotometer RF-1501). The present study proposes a new microoptical method that excites at the wavelength of 405 nm but measures the fluorescence emission ratios between the red (>590 nm) /green (510-590 nm) and the red/blue (450-510 nm) wavelengths, respectively.

Material & Methods

We compared the detection of PPIX with conventional fluorescence spectroscopy and the new optical probe in three cultured transitional cell carcinoma (TCCs) lines (VmCub 1, SW1710, 5637) incubated with 5-ALA. Cells were grown on glass slides and were incubated with 2 mM 5-ALA at 37°C for 6 hours. Controls were incubated in the absence of 5-ALA. At one-hour intervals, cells were washed twice with DMEM and covered with PBS. The fluorescence emission of PPIX was measured in three samples and one control using excitation and single emission for conventional fluorescence spectroscopy and the multiple wavelength emission ratios for the microoptical probe. After 4 hours of incubation with 5-ALA we performed scans with the newly developed microoptical probe with 1mm stepwidth across the glas slide. For this experimental setup we used the most malignant cell line 5637. The results show that also thin cell layers of malignant cells can be detected with this method and a distinction between normal and cancerous tissue seems to be possible (see fig.5).

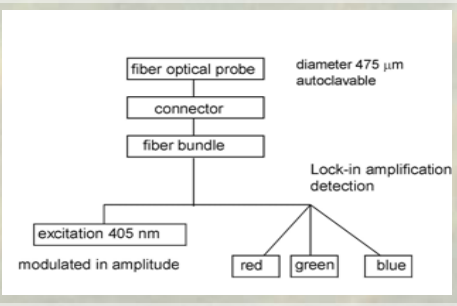

fig. 1 setup microoptical probe

Results

The results from conventional fluorescence spectroscopy demonstrated that, as the incubation time increases, the amount of endogenously produced PPIX rises.

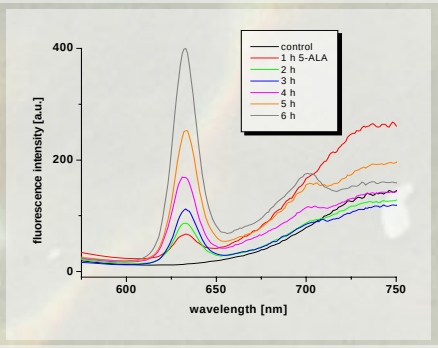

fig. 2 fluorescence emission spectra of 5637 cell monolayer on glass slide incubated with 2 mM 5-ALA (excitation wavelength 405 nm)

Considering the following results for all cell lines, there is a linear rise in fluorescence intensity at 633 nm excited at 405 nm dependent on the incubation time with 5-ALA (see fig. 3a-c).

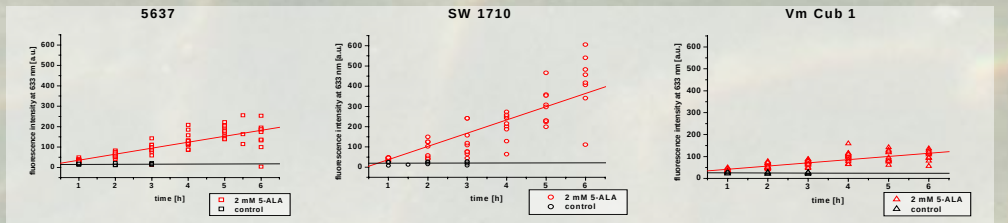

fig. 3a-c red fluorescence emission at 633 nm after incubation with 2 mM ALA (red symbols) and controls (black symbols)

Also in case of the microoptical probe the ratio between red and green fluorescence rises in linear way in favour of the red fluorescence. After 3 hours of incubation, a clear distinction between the control fluorescence and the 5-ALA treated cells was noted. Kinetics of endogenously produced PPIX in the different cell lines are presented in table 1.

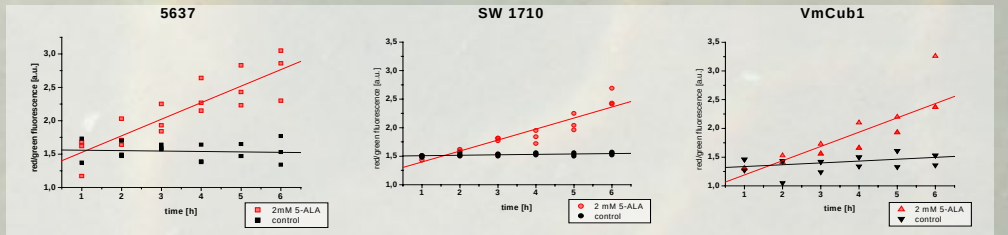

fig. 4a-c red fluorescence emission at 633 nm after incubation with 2 mM ALA (red symbols) and controls (black symbols)

	Fluorimeter		microoptical probe	
Cell line	**633 nm**	**control**	**Red/green**	**control**
5637	29,26667	0,493	0,24829	-0,0066
VmCub 1	14,61923	-0,4028	0,19286	0,00743
SW 1710	38,84478	0,3324	0,24857	0,03171

table 1
kinetics of endogenously produced PPIX for all three cell lines [a.u./h]

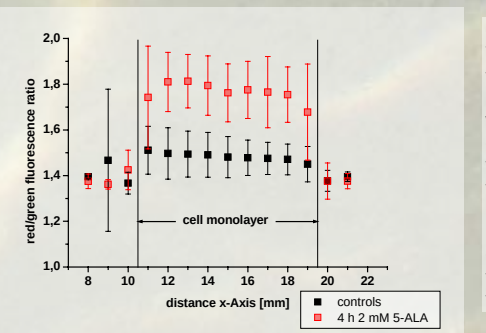

fig.5 scan with the microoptical probe across a cell monolayer of 5637 bladder arcinoma after 4 h 2 mM 5-ALA (red symbols) and controls (black symbols)

Conclusions

The findings indicate that this new tool for optical biopsy in the context of PDD could be useful for detection of (pre-) malignant alterations, especially in the urogenital tract. It would be possible to insert the device through an endoscope into hollow-organ-systems, and so it should be tested *in vivo* as a more informative diagnostic alternative than the conventional fluorescence endoscopy.

Moreover, the results demonstrate that the new microoptical probe is able to detect even a thin monolayer of malignant cells, so that it seems probable to have a new opportunity to find flat malignant lesions which are difficult to distinguish from the intact urogenital epithelium.

Literature

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