Results in Physics 8 (2018) 1232–1233

Contents lists available at ScienceDirect

Results in Physics

journal homepage: www.journals.elsevier.com/results-in-physics

Fiber-optic filter fluorometer for emission detection of Protoporphyrin IX and its direct precursors – A preliminary study for improved Photodynamic Therapy applications

Rainer Landes [a,*], Alfredo Illanes [a], Alexander van Oepen [a], Daniela Goeppner [b], Harald Gollnick [c], Michael Friebe [a]

[a] Otto-von-Guericke University, Roetgerstr. 9, Magdeburg 39104, Germany
[b] Justus-Liebig-University, Gaffkystr. 14, Giessen 35385, Germany
[c] University Hospital Magdeburg, Leipziger Str. 44, Magdeburg 39120, Germany

ARTICLE INFO

Article history:
Received 18 October 2017
Received in revised form 3 January 2018
Accepted 23 January 2018
Available online 31 January 2018

2000 MSC:
41A05
41A10
65D05
65D17

Keywords:
Photodynamic Therapy
Drug light interval
Protoporphyrin IX
Coproporphyrin III
Uroporphyrin III

ABSTRACT

In this work we present first results of a laboratory manufactured filter-fluorometer to study differences in intensity and position of the main peaks of three porphyrins that appear during the Heme-Synthesis. Porphyrins play a major role in Photodynamic Therapy (PDT) for cancer treatment. Within the Heme-Synthesis, Porphyrins such as Protoporphyrin IX (PPIX) and its two precursors Coproporphyrin III (CPIII) and Uroporphyrin III (UPIII) represent photochemical agents that can interact with light to show fluorescence or generate Reactive Oxygen Species (ROS) to destroy cells. A major problem that arises is determining the ideal time slot to begin treatment after drug application. Our work is meant to show a way to solve this problem by looking at concentration changes of precursors appearing in Heme-Synthesis and using these changes to predict the occurence of PPIX inside the mitochondria.

Introduction

Photodynamic Therapy (PDT) consists of a photochemical reaction that involves three main components: a photosensitizer (PS), oxygen and light with an appropriate wavelength. Although a positive response was observed for nearly all types of solid tumors, unpredictably high recurrence rates mean that PDT still needs additional improvement, as the complex and still hardly understood dynamical relationship between the involved variables makes the process difficult to monitor and optimize [1]. A PDT process starts with the transport of PS inside the tumor, either by exogenously injecting the PS directly into the targeted tissue or by inducing an endogenous process for the generation of the PS [1]. The latter allows an optimal tumor selectivity using aminolevulinic acid (ALA), which gradually builds photoactive molecules inside the tumor cells [2]. The PS mainly targeted by this approach is Protoporphyrin IX (PPIX) [3]. In order to achieve the highest possible impact on the cancer cells and destroy them the position of the PS inside the cells is of high importance [4]. For this reason we chose to study the transformation process of ALA to PPIX, since in the metabolic pathway producing PPIX there appear two other porphyrins which are built outside the mitochondria. A prerequisite to localize and study these last steps of the process is to be able to distinguish PPIX and these precursors to measure changes [5]. Literature values give positions for the main emission peaks of PPIX, after excitation with light at 405 nm in the range of 630–640 nm and for CPIII and UPIII more than 10 nm lower [6,7]. The peak of the two precursors can always be expected to be at least 10 nm lower than the peak of PPIX. To conduct this study we built a fiber-optic filter fluorometer (Fig. 1).

* Corresponding author.
E-mail address: rainer.landes@ovgu.de (R. Landes).

https://doi.org/10.1016/j.rinp.2018.01.059
2211-3797/

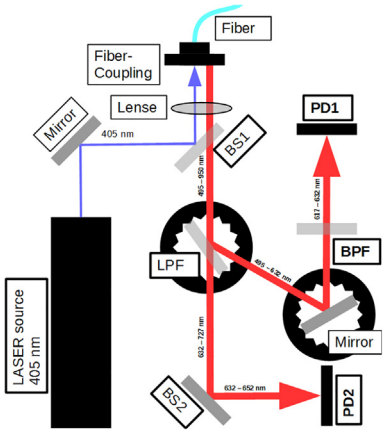

Fig. 1. Schematic view of the Filter-Fluorometer with the tunable Longpass Filter (LPF) set to 632 nm, the blue arrowed line representing the beam out and the red arrowed line representing the received fluorescence. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

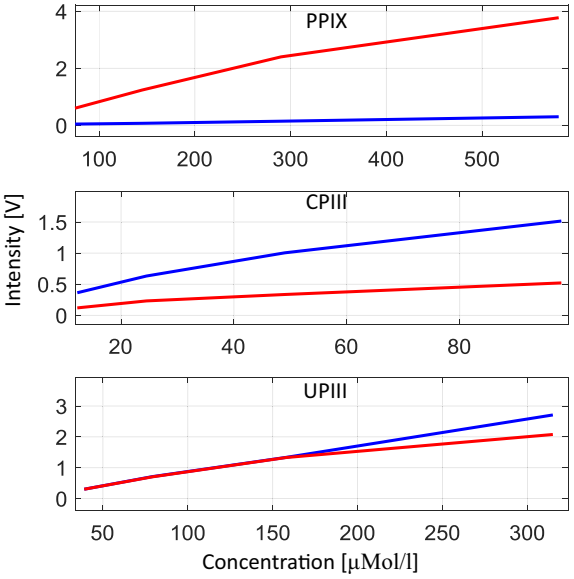

Fig. 2. Comparison of pure solutions of all three Porphyrines in dilution series, red dashed line: signal received by Photodiode 2, representing the higher wavelength range, Blue line: signal received by Photodiode 1, representing the lower wavelength range. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Materials and methods

The optical setup for the filter fluorometer consists of a laser (PPM110 (405–125), Power Technology, USA) for excitation and several filters which were bought from AHF analysentechnik AG, Germany (Table 1). Phantoms were liquid with a 20 percent Methanol/80 percent PBS composition and the pH was adjusted to 7,4 with HCl or NaOH respectively. The two precursors UPIII and CPIII could only be purchased as methyl esters, unsoluble in aqueous solution and had to be treated by alkaline hydrolysis to make them water soluble [8].

Each solution was measured for 120 s by placing the fiber centrally inside the phantom and irradiating the sample. The fluorescence emission was channeled back to the fluorometer and the spectral range was filtered. For this purpose the tunable filter was set to an edge at 632 nm, which is closer to the expected position of the PPIX peak and should be well above the peaks of the two precursors. With this setting the photodiodes received a lower range from 607–632 nm (PD1) corresponding to fluorescence belonging to the precursors and an upper range 632–652 nm (PD2) corresponding to PPIX fluorescence.

Results and discussion

Our goal for this very first proof of concept was to show that Protoporphyrin IX and the two precursors UPIII and CPIII can be distinguished by the position of the peaks in the wavelength range from 600–650 nm. This goal was successfully achieved (Fig. 2). Important is the change of intensity from Photodiode 2 (red line) to Photodiode 1 (blue line) when changing from PPIX samples to precursor samples. Differences in concentration between samples of different porphyrins are of no concern for this approach, since only changes within one set of samples should be detectable. These changes signify the position of the main peak in the upper wavelength range for PPIX and in the lower wavelength range for CPIII and are consistent at different levels of dilution within each set of samples. The behaviour of the signal detected for UPIII, with both Photodiodes receiving almost the same signal intensity shows that the main peak of UPIII must be located closer to the position of the edge separating the upper and the lower wavelength range. The change of intensity between the lower and upper range shows the last steps of the Heme-Synthesis. An increasing signal in the lower range will predate a change in the upper range and therefore an increase in PPIX concentration. For in vivo applications in the future this will be used to determine the optimal Drug-Light Interval which will increase the efficiency of the therapy.

Table 1
Main components used for the optical setup were all bought from Semrock, New York, USA.

BS1	FF495-Di03	reflection: <495 nm transmission: >495 nm
LPF	TLP01-704	tunable: 617–704 nm
BPF	FF02 632/22	Band Pass: 607–635 nm
BS2	FF650-Di01	reflection: <650 nm transmission: >650 nm

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, athttps://doi.org/10.1016/j.rinp.2018.01.059.

References

[1] Agostinis P et al. Photodynamic therapy of cancer: an update. CA: Cancer J Clinicians 2011;61:250–81.
[2] Wang Wenqian et al. Enhancement of 5-aminolevulinic acid-based fluorescence detection of side population-defined glioma stem cells by iron chelation. Sci Rep 2017:7.
[3] Choudhary Sonal KN, Elsaie ML. Photodynamic therapy in dermatology: a review. Lasers Med Sci 2009;24:971–80.
[4] Liu Hong-Wen et al. An efficient two-photon fluorescent probe for monitoring mitochondrial singlet oxygen in tissues during photodynamic therapy. Chem Commun 2016;52:12330–3.
[5] Inoue et al. Massive apoptotic cell death of human glioma cells via a mitochondrial pathway following 5-aminolevulinic acid-mediated photodynamic therapy. J Neuro-Oncol 2007;83(3):223–31.
[6] Deacon E. Front line tests for the investigation of suspected porphyria. ACP Best Pract 2001:165.
[7] Montcel B et al. Two-peaked 5-ala-induced ppix fluorescence emission spectrum distinguishes glioblastomas from low grade gliomas and infiltrative component of glioblastomas. Biomed Opt Express 2013;4(4):548.
[8] Karmakar A, G.I. A. Flexible porphyrin tetracarboxylic acids for crystal engineering. Cryst Eng Commun 2010;12(12).