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Application of the Z-scan technique for the detection of CFCDNA (cell-free circulating DNA) and urine DNA (uDNA) in patients with bladder cancer

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ABSTRACT

Background: Patients with BC have a higher amount of cell-free circulating DNA (CFCDNA) in the blood and urine than healthy people. We aimed to verify if the Z-Scan method could analyze the concentrations of uDNA (urinary) and pDNA (plasma) in relation to the time of collection during treatment for patients with bladder cancer.

Methods: Peripheral blood and urine samples were obtained from 30 patients with BC at the time of diagnosis, 45, 90 and 180 days after initiating treatment; 5 μL of k-DNA (k = u or p) was added in 250 μL a solution of 1:1000 Ethidium Bromide dye (EtBr) in water. Continum laser Nd:YVO4, wavelength λ = 532 nm was used. Samples of uDNA and pDNA in water were submitted to the laser with an incident power of 84.5 mW and an exposure time of 30 ms.

Results: There was a different concentration of pDNA and uDNA during the treatment of patients using both optical techniques. However, the reaction rate of pDNA and uDNA was similar with spectrophotometry, whereas the z-scan technique presented different values.

Conclusion: Z-scan technique has potential for use in the differentiation of pDNA and uDNA concentrations, which are distinct in patients with BC and healthy people.

1. Introduction

The second most common cancer of the urinary system is of the bladder. Three types of Bladder Cancer (BC) are defined according to cellular mutations: 1. Transitional cell carcinoma, which begins in the inner tissues of the bladder, spreads through the lining of the bladder, affects the muscular wall and invades nearby organs and/or lymph nodes, and is therefore invasive; 2. Squamous cell carcinoma, which occurs after infections and prolonged irritations in the thin and flat cells of the bladder; 3. Adenocarcinoma associated with secretory glandular cells, which are also due to irritations or inflammation. If the BC is confined to the bladder lining, it is defined as superficial cancer [1].

BC diagnostic methods are by urine and imaging tests, such as computed tomography and cystoscopy, during which a biopsy or total tumor removal can be performed and cure probability depends on the stage in which diagnosis occurs [2]. Patients with BC have a higher amount of cell-free circulating DNA (CFCDNA) in the blood and urine than patients without cancer, one of the reasons underlying metastatic degeneration, compromising the patient's life [3,4]. Cell-free circulating DNA can originate from cell apoptosis or necrosis of solid tumors and may serve as a biomarker for tumor detection and follow up [5].

The non-linear optical technique, Z-Scan , has been used for the study of biological fluids [6–8] and in diverse materials due to the sensitivity and precision of the technique [9]. The high intensity of the beam allows for intense electric fields interacting with the matter, altering the medium and the light beam. The Z-Scan technique analyzes these alterations, via the signal and the magnitude of the refractive index, using a simple relationship between the observed variation in

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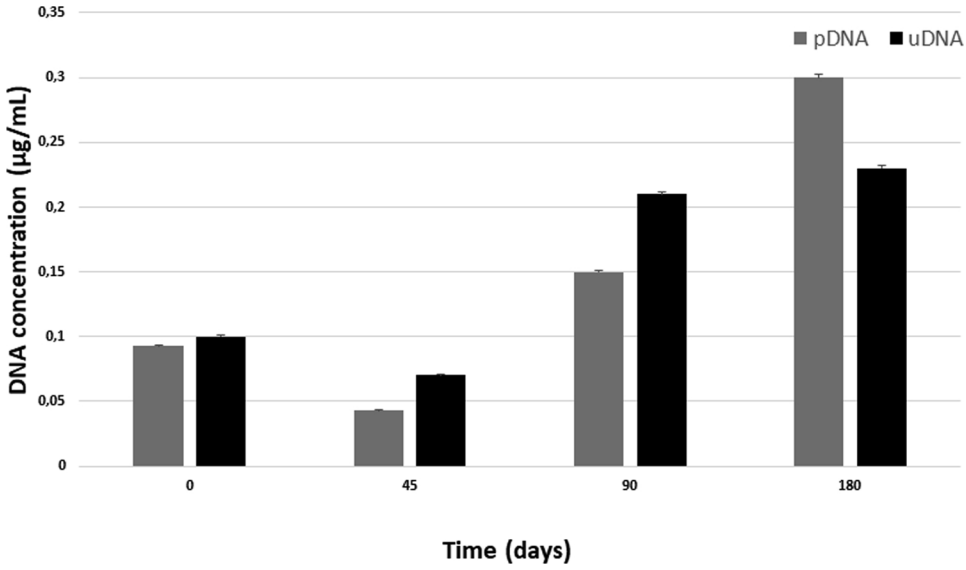

Fig. 1. Concentration of DNA (µg/ mL) of cancer patients. Anova Test: $p < 0.001$; 95% IC.

Table 1
Absorption values of uDNA and pDNA sample at $\lambda = 532$ nm.

Time (days)	Plasma (cm^{-1})[a]	Urine (cm^{-1})[a]
0	0.104	0.103
45	0.099	0.105
90	0.102	0.102
180	0.104	0.088

[a] For both parameters, mean ± 0,005 cm^{-1} values were shown.

transmittance and the induced phase distortion.

The Z-scan technique stands out for having a greater optical sensitivity than the usual laboratory methodologies, differs from others arising from linear optics such as spectroscopy and fluorescence and is characterized by the simplicity of its experimental arrangement [7].

For this work, we aimed to verify if the Z-Scan method is able to analyze the concentrations of uDNA (urinary) and CFCDNA (plasma) in relation to the time of collection during treatment for patients with bladder cancer.

2. Patients and methods

2.1. Samples

Peripheral blood and urine samples were obtained from 30 patients, who signed the Free Informed Consent, approved by the Ethics Committee of the University Center of Medical Sciences of ABC, with BC at the time of diagnosis 0, 45, 90 and 180 days after initiating treatment.

For sediment analysis and DNA extraction from urine samples, in addition to laboratory, clinical analysis and DNA extraction from plasma DNA samples, the reference extraction method (de Almeida et al. [4]) was used.

5 µL of k-DNA (k = u or p) was added in 250 µL a solution of 1:1000 Ethidium Bromide dye (EtBr) in water. EtBr was used as a doping to enable the study of DNA at 532 nm by the Z-Scan technique. Ethidium Bromide dye is a double-strand intercalating dye routinely used in visualizations of genetic material samples in molecular biology techniques, which can be detected with a laser wavelength of 532 nm.

The choice of EtBr was due to the optical effects of the dye in the Z-Scan technique being known and already having been studied, and the signal noise of the analyzed samples was eliminated.

2.2. Z-scan

The experimental sketch can be found in Gomez et al. [6]. Continum laser Nd:YVO_4 (Verdi 2 -Coherent®), wavelength $\lambda = 532$ nm was used.

The samples of uDNA and pDNA in water were conditioned in 200 µm-thick glass samples and submitted to the laser with an incident power of 84.5 mW and an exposure time of 30 ms.

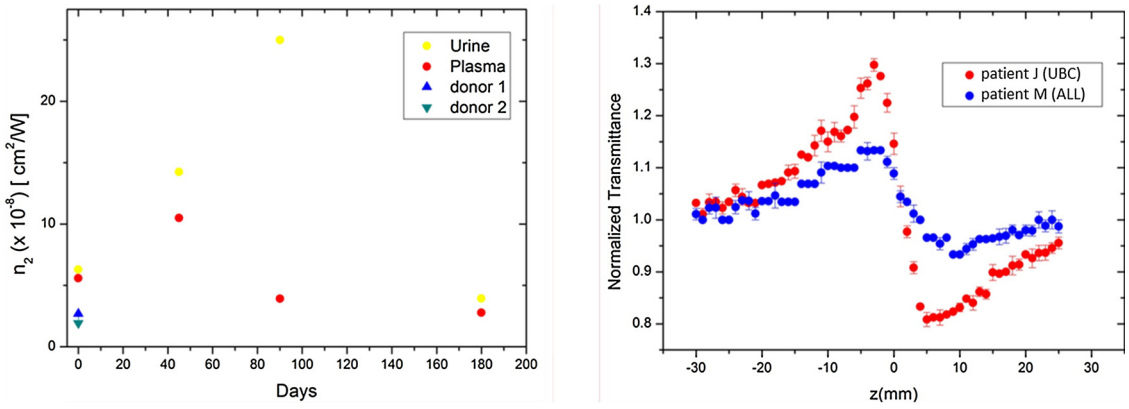

Fig. 2. a) Z-scan reading of a uDNA and pDNA sample. Circle: uDNA; Triangle: pDNA; black star: donor 1; open star: donor 2 and b) The difference in signal readings from samples obtained from a patient with BC (circle) and another with leukemia (triangle).

2.3. Statistical analysis

For the statistical analysis of measurements, the SPSS® 18 program was used. Data were expressed as Mean ± SD. Differences between the collections were analyzed by the analysis of variance (One-way ANOVA). Only p values < 0.05 were considered statistically significant.

3. Results and discussion

The difference in the concentrations of uDNA and pDNA, respectively, of the patients during treatment noted by spectrophotometry can be found in Fig. 1. Table 1 shows the absorption values of uDNA and pDNA sample at $\lambda = 532$ nm; this wave-length was choose because the ethidium bromide absorption ($0.109\,cm^{-1}$) after some previous results of Z-scan technique was already known.

Fig. 2 shows results obtained with Z-scan technique in a) Z-scan reading of a uDNA and pDNA sample and b) the difference of sample signals from a patient with BC and one with leukemia. As can be seen, N2 (refractive index) increases until 45 days, when it targeted its peak and was reduced and its minimum after 180 days of the treatment (uDNA and pDNA). In 90 days, N2 of uDNA but not pDNA was increased; the same result could be observed by spectrophotometry technique.

4. Conclusion

There was a difference in the concentration of pDNA and uDNA during the treatment of patients using both optical techniques. However, the reaction rate of pDNA and uDNA was similar with spectrophotometry, whereas the z-scan technique presented different values. Finally, the z-scan technique has potential for use in the differentiation of pDNA and uDNA concentrations, which are distinct in patients with BC and without carcinomas.

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