UV ABSORBANCE OF LYMPHOCYTES

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ABSTRACT

The UV absorption spectra of human lymphocytes were obtained. It was found that UV absorption spectra of lymphocytes from blood of patients with B-cell chronic lymphocytic leukemia have a noticeable peak with maximum at ~260 nm, and spectra of lymphocytes of healthy subjects do not have such a peak. Since the spectra obtained are similar to UV absorption spectra of nucleic acids, we concluded that the UV absorption of lymphocytes is mainly due to the absorption of lymphocyte nucleic acids. Furthermore, after comparison the spectra obtained with the spectra of the oxidized and not oxidized nucleic acids, we convinced that a peak value at 260 nm reflect the degree of oxidation of the lymphocyte nucleic acids. In this fashion we concluded that the root cause of chronic lymphocytic leukemia is the oxidation of lymphocyte nucleic acids. This conclusion explains why the UV absorption spectra of lymphocytes of healthy subjects do not have the peak at ~260 nm – their nucleic acids are little oxidized. In the end we came to the conclusions: 1. Nucleic acids can be oxidized at their isolation – such oxidation results in the appearance of peaks at 260 nm in their UV absorption spectra. 2. UV absorption spectra of nucleic acids healthy organisms may not have such a peaks.

Keywords: Blood, lymphocytes, DNA, RNA, UV absorption spectra.

INTRODUCTION

The study of whole blood by UV spectroscopic techniques can be used both for the diagnosis of the disease and for understanding the biological nature of the disease ([a]Gunasekaran at al., 2008; [b]Gunasekaran at al., 2008; Kanagathara at al., 2011). However, whole blood is very complex fluid. So the UV absorption spectra of blood are too difficult to detail analyzes. For that reason we found it necessary to study the UV absorption spectra of the individual blood fractions. Therefore we studied the UV absorption spectra of lymphocyte fractions. Namely, we examined the UV absorbance of lymphocytes of healthy subjects and patients with B-cell chronic lymphocytic leukemia (B-CCL). Here we offer our results.

METHODOLOGY

The working solutions of lymphocytes were obtained in correspondence with methodic of institute of experimental pathology, oncology and radiobiology NAS Ukraine. Blood samples of healthy subjects were collected from healthy volunteers of different age groups. Blood samples of patients with B-CCL also were collected. The lymphocytes were separated from every sample. The samples were centrifuged at a speed of 1500 rpm in density gradient of Ficoll-Urographin. The lymphocytes were washed by Hanks` Balanced Salt Solution (HBSS).The washed lymphocytes were dissolved in HBSS. These working solutions contained 10^6 cells/ml. The UV absorption spectra of lymphocyte working solutions were recorded through 15 – 30 min. after their preparation; ~90% of cells remained alive, during recording. To UV spectra recording Specord UV VIS (Carl Zeiss Jena, Germany) was used.

RESULTS

It was found that, in opposed to UV absorption spectra of lymphocytes from healthy subjects (fig. 1, spectrum 1), the UV absorption spectra of lymphocytes from patients with B-CCL have a noticeable peak at ~260 nm (Figure 1, spectrum 2).

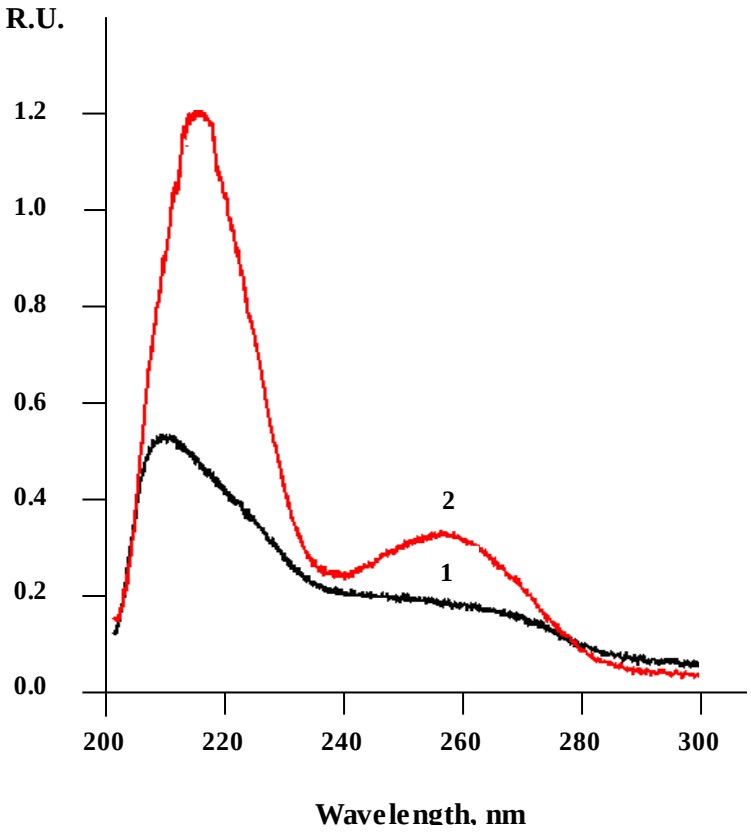

Figure 1. UV absorption spectra of lymphocytes. 1 – lymphocytes of healthy subjects: 2 – lymphocytes of patients with B-CCL.

The difference between these spectra was observed also in range 200 – 220 nm: the UV absorption spectra of lymphocytes from healthy subjects have a peak at 208 nm (Figure 1, spectrum 1) and the spectra of lymphocytes from patients with B-CCL – a peak at 215 nm (Figure 1, spectrum 2).

DISCUSSION

Since the UV absorption spectra of lymphocytes of patients with B-CCL have the noticeable peak at ~260 nm (Figure 1, spectrum 2), in opposed to UV absorption spectra of lymphocytes from healthy subjects (Figure 1, spectrum 1), we concluded that these spectra can be used to diagnose of B-CCL. We also concluded that the observed spectral difference helps to understand the underlying biological causes of the disease.

It is known that in lymphocytes of B-CCL patients is an active RNA synthesis (Silber at al., 1968). That is, the appearance of a significant peak at ~260 nm in the UV absorption spectra of lymphocytes of examined patients (Figure 1, spectrum 2) can have obvious explanation. However, after analysis of the literature, we concluded that the obtained spectra (Figure 1) are similar to the UV absorption spectra of oxidized and non-oxidized DNA (Khan at al.,

2006; Doshi at al., 2009; Doshi at al., 2010; Pivovarenko, 2015). We also observed the similar changes for the UV absorption spectra of DNA (Pivovarenko, 2014) and RNA stored in degassed solutions (Figure 2).

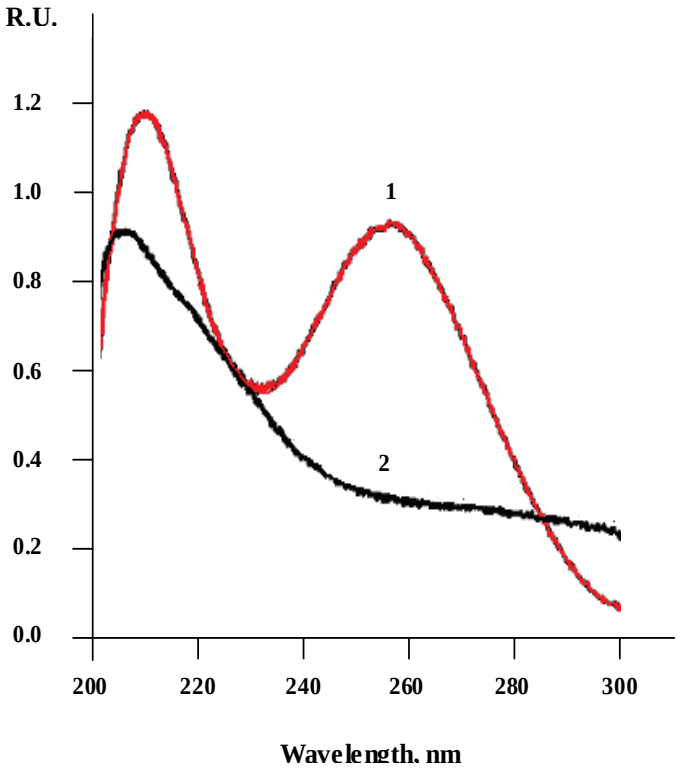

Figure 2. UV absorption spectra of RNA degassed solutions: 1 – freshly prepared RNA solution; 2 – the same RNA solution after storage.

On the basis of this similarity, we made two assumptions:
1. The UV absorption of lymphocytes is due mainly to the absorption of lymphocyte NAs;
2. The lymphocytes NAs of patients with B-CCL are more oxidized than NAs of healthy subjects.

In our view, these assumptions are well explained by the difference between the spectra obtained (Figure 1). It is important that they explain the difference observed in the wavelength range 200 – 220 nm (Figure 1, spectra 1,2). Such a difference cannot be explained only by the greater concentration of RNA in the lymphocytes of patient's organisms (Silber at al., 1968). Besides that these assumptions correlate with the established facts:
1. Oxidative damages of macromolecules can activate transcription (Pratviel at al., 1995);
2. Oxidative damages of DNA can result in cancer (Poulsen at al., 1998; Khan at al., 2005).

In addition, our explanations are well agreed with the fact that oxidation of DNA is the factual cause of many other diseases (Frenkel, 1992; Esterbauer at al., 1992; Esterbauer at al., 1992; Markesbery, 1997; Cooke at al., 2003).
Finishing, we want to formulate a problem. Since UV absorption spectra of NA working solutions typically have a peak at ~260 nm, it can be concluded that dissolved NAs oxidized. (Most likely, they are oxidized for the period of isolation.) So long investigated only oxidized NAs. Therefore, we have a lot of information about the properties of oxidized NAs and have

no information about the properties of non-oxidized NAs. That is, we have a lot of information about the properties of NAs from patient's organisms, but we have no information about the properties of NAs healthy organisms. Agree that it is discouraging.

CONCLUSIONS

UV absorption spectra of lymphocytes from blood of patients with B-cell chronic lymphocytic leukemia have a noticeable peak with maximum at ~260 nm, and spectra of lymphocytes of healthy subjects do not have such a peak. Is the spectral difference can be used for diagnosis of B-cell chronic lymphocytic leukemia.

The UV absorption of lymphocytes is mainly due to the absorption of lymphocyte nucleic acids. The lymphocytes NAs of patients with B-CCL are more oxidized than NAs of healthy subjects.

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