

JURKAT E 6.1 CELL LINE STUDIES REGARDING THE EFFECTS OF SOME BIO-INDOLS ON THE MEMBRANE FLUIDITY

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Abstract

Many recent research studies have reported that membrane fluidity is highly affected by oxidative stress, namely this mechanism has been strongly correlated with the rigidization of the membrane. It has been demonstrated that chronic diseases that exhibit high oxidative stress (Alzheimer, Parkinson, diabetes, cancer) are characterized at the membrane level by an important diminishing of the membrane fluidity (rigidity). Therefore, finding medicines that increase the membrane fluidity may constitute an important therapeutic resource for oxidative stress diseases at the cellular level.

The aim of the study was the assessment of the potential modifications of the membrane fluidity induced by some bio-indols, namely melatonin, serotonin, and tryptophan.

We used an *in vitro* experimental model on Jurkat E 6.1 lymphoblast cell line, and we assessed the effect of the studied bio-indols on membrane fluidity, using a polarized fluorescence analysis system.

Rezumat

Cercetări recente au demonstrat că fluiditatea membranară este puternic influențată de către stresul oxidativ, acest proces fiind corelat cu rigidizarea membranară. Studii științifice susțin faptul că bolile cronice, care presupun niveluri crescute ale stresului oxidativ (Alzheimer, Parkinson, diabet, neoplasm), sunt caracterizate prin scăderea fluidității membranare (rigidizare). În consecință, orice biomoleculă sau compus de sinteză care stimulează fluiditatea membranară poate constitui o resursă de mare interes terapeutic pentru afecțiunile determinate de stresul oxidativ, la nivel celular.

Scopul acestui studiu a fost evaluarea modificărilor anizotropiei (și, deci, fluidității) membranare induse de unii bio-indoli: melatonina, serotonina și triptofanul.

S-a utilizat ca model linia celulară limfoblastică Jurkat E 6.1 și s-a determinat efectul bio-indolilor utilizați asupra fluidității membranare, folosind un sistem de analiză cu fluorescență polarizată.

Keywords: bio-indols, melatonin, Jurkat E 6.1 lymphoblast cell line, membrane fluidity, polarized fluorescence analysis, oxidative stress

Introduction

The cellular membrane is a relatively stable structure that lasts for the entire cell life-cycle, in spite of the fact that its components undergo permanent refurbishments and modifications. It has a far more complex role than participating to the intra-extracellular transport of different constituents, as it facilitates the cell active participation to morphogenesis and recognition processes [2,3,6,8].

Alternations in cellular lipid composition and trafficking due to oxidative stress will affect the activity of the intracellular biochemistry, because the lipid membrane components possess specific rotational and lateral diffusion rates that allow the physiological transmembranar transport. This specific membranar process is known in the scientific literature as „the membrane fluidity”. Many recent research studies have reported that membrane fluidity is highly affected by oxidative stress, namely this mechanism has been strongly correlated with the rigidization of the membrane.

It has been demonstrated that chronic diseases that exhibit high oxidative stress (Alzheimer, Parkinson, diabetes, cancer) are characterized at the membrane level by an important diminishing of the membrane fluidity (rigidity). Therefore, finding medicines that increase the membrane fluidity may constitute an important therapeutic resource for oxidative stress diseases at the cellular level [9,11,12].

At the moment, there have been identified only few compounds that can antagonize the membrane rigidization process, that can in accordance stimulate the membrane fluidity (e.g. general anesthetics, quercetin).

One of the major concerns of modern pharmaceutical research is finding therapeutic resources (drugs) with high efficiency and diminished or absent adverse effects. Biological therapies (the discovery and retrieval of endogenous resources of the human organism) represent, at the moment, one of the most thoroughly scientifically argued domains. In this acceptance, the physio-pathological and pharmacological potential of the bio-indols (melatonin, serotonin, tryptophan) represents the subject of modern research regarding the organisms adaptive integration in the great informational diversity of the exterior surrounding environment [10,14].

The aim of the study was the assessment of biochemical mechanisms of some bio-compounds with indolic structure, by the analysis of their biochemical effects on membrane fluidity. The potential modifications of the membrane fluidity induced by the studied bio-indols, may be further used for improving the pharmacotherapy of diseases characterized by alterations of this specific membrane parameter.

On these lines, we used an *in vitro* experimental model, and we assessed, on culture cells, the membrane fluidity.

Materials and Methods

The experimental research was carried out in the Biochemistry Department, of the Faculty of Pharmacy, Bucharest, Romania, using as experimental model a Jurkat E6.1 lymphoblastoma cell line, primary and secondary culture obtained at the “Victor Babeş” National Institute of Research and Development in the Field of Pathology and Biomedical Sciences, Bucharest (figure 1). The culture medium used for obtaining the cellular suspensions was RPMI-1640 (*Roswell Park Memorial Institute medium*) supplemented with L-glutamine; the solution was refrigerated at 4°C. There were obtained:

- a) the primary culture: $3-9 \times 10^5$ cells/mL in RPMI-1640 culture medium supplemented with fetal bovine serum (FBS) (10%), 2 mM L-glutamine; the culture was maintained for 3 days at 37°C, in controlled atmosphere (95% air, 5% CO₂);
- b) the secondary culture: was obtained by replacing the culture medium and the cells resuspension in RPMI– 1640, to a density of 10^5 viable cells/mL.

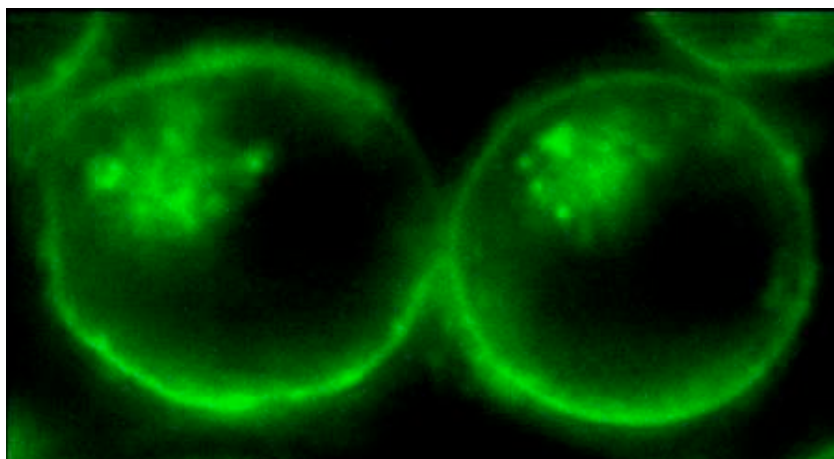


Figure 1

Microscopic image of the Jurkat lymphoblasts

Reagents: RPMI 1640 medium was purchased from Promega, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene-p-toluenesulfonate (TMA-DPH) was obtained from Molecular Probes; Melatonin, Serotonin, Tryptophan were purchased from Sigma-Aldrich.

Fluorescent probes: for evaluating the membrane anisotropy it was used a phenyl hexatriene probe: 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene-*p*-toluenesulfonate (TMA-DPH). We obtained a 2.5 mM stock solution in dimethylsulfoxide (DMSO), maintained at -20 °C.

Chemical stimuli: melatonin, serotonin and tryptophan solutions of the concentrations: 1 μM, 2,5 μM, 5 μM, 10 μM, 20 μM, 25 μM, were prepared in the culture medium RPMI-1640.

Equipment: LS50 B spectrofluorimeter Perkin Elmer, equipped with thermostated cell holder, magnetic stirring and fluorescence polarization accessory.

Fluorescence anisotropy evaluation

The fluorescence anisotropy of TMA-DPH incorporated in the cells was assessed by the determination of steady state fluorescence polarization of the membrane-fluorescent probe system; the probe (TMA-DPH) lacks fluorescence in solution and becomes fluorescent when incorporated into the lipid membrane bilayer.

We first evaluated the basic autofluorescence of the Jurkat cell suspension. For the measurement of the changes in the TMA-DPH fluorescent properties following the membrane insertion, we added to a 2 mL of Jurkat cell suspension an aliquot of TMA-DPH stock solution in DMSO to get a 2.5 μM TMA-DPH in the measurement cuvette. The cell suspension with the fluorescent probe was incubated for 2 minutes at 37°C under continuous magnetic stirring. Steady state fluorescent polarization of TMA-DPH was further measured; the TMA-DPH was excited with polarized light at 355 nm and the emission intensities were detected at 430 nm, through another polarizer system. We incubated each sample with the chemical stimuli (melatonin, serotonin and tryptophan solutions of different concentrations) for 20 minutes at room temperature, and then we measured again the TMA-DPH fluorescent properties. Calculation of the fluorescence anisotropy (r) was performed according to the equations (1) and (2):

$$r = \frac{I_{vv} - GI_{vo}}{I_{vv} + 2GI_{vo}} \quad (1) \quad ; \quad G = \frac{I_{ov}}{I_{oo}} \quad (2)$$

where r is the fluorescence anisotropy, I_{vv} , I_{vo} , I_{ov} and I_{oo} represent the emission intensity corrected for the autofluorescence signal of unstained cells, when the polarizers in the excitation and emission beams are oriented in vertical-vertical, vertical-horizontal, horizontal-vertical and horizontal-horizontal positions, respectively [6,12].

Statistical analysis

Each sample was assessed 10 times, so the final value of each sample is the mean $\pm$ SD of 10 determinations. Statistical significance was determined using the SPSS software. Significance was set at $p \leq 0.05$.

Results and Discussion

In table I and figure 2 there are presented the experimental results that depict the dynamics of the fluorescence membrane anisotropy under the effect of the studied endogenous indols (melatonin, serotonin, tryptophan).

Table I

The dynamics of the fluorescence membrane anisotropy under the effect of the studied bio-indols

Indol concentration	The fluorescence anisotropy of melatonin	The fluorescence anisotropy of serotonin	The fluorescence anisotropy of tryptophan
Blank (without stimuli)	0.415 $\pm$ 0.033	0.392 $\pm$ 0.029	0.431 $\pm$ 0.038
1 μ M	0.241 $\pm$ 0.035	0.371 $\pm$ 0.037	0.442 $\pm$ 0.044
2,5 μ M	0.223 $\pm$ 0.029	0.389 $\pm$ 0.032	0.445 $\pm$ 0.039
5 μ M	0.219 $\pm$ 0.028	0.386 $\pm$ 0.035	0.427 $\pm$ 0.039
10 μ M	0.205 $\pm$ 0.031	0.422 $\pm$ 0.036	0.415 $\pm$ 0.038
20 μ M	0.187 $\pm$ 0.032	0.414 $\pm$ 0.037	0.424 $\pm$ 0.042
25 μ M	0.157 $\pm$ 0.034	0.402 $\pm$ 0.033	0.431 $\pm$ 0.043

The experimental results, mathematically processed according to equations 1 and 2, revealed important information regarding the effect of the studied indolic bio-molecules on the cell membrane fluidity.

In this regard, the pineal hormone registered the lowest values of membrane anisotropy, so the highest rates of the lipid bilayer membrane fluidity. The obtained data were statistically significant (lower values) for melatonin, compared to serotonin ($p < 0.001$) and tryptophan ($p < 0.001$).

At the 10 μ M concentration, melatonin registered a 0.205 $\pm$ 0.031 fluorescence anisotropy, while serotonin (0.422 $\pm$ 0.036) and tryptophan

(0.415 ± 0.038) registered anisotropy levels similar to samples without stimuli (0.392 ± 0.029 and respectively 0.431 ± 0.038).

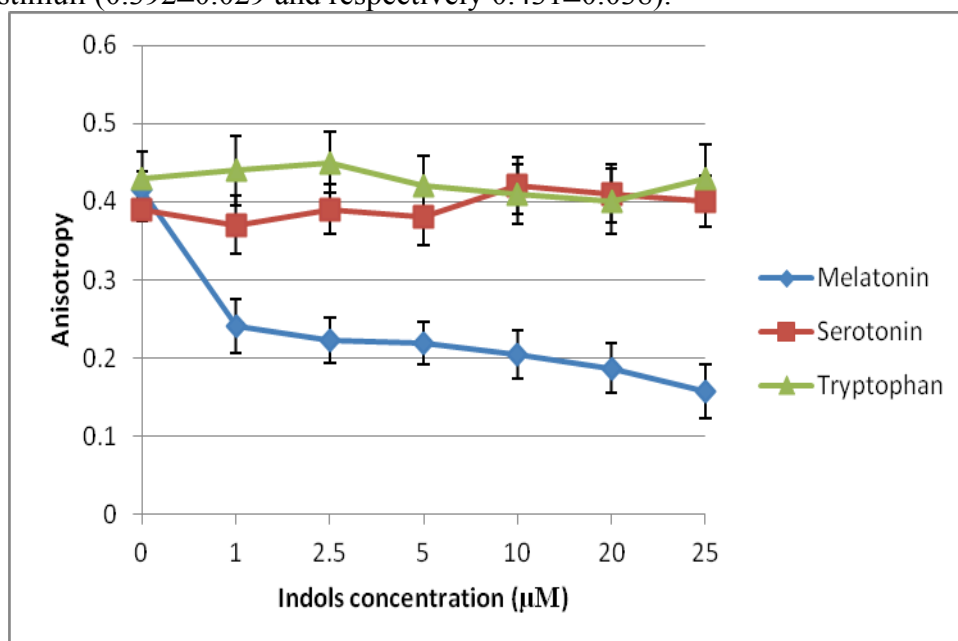


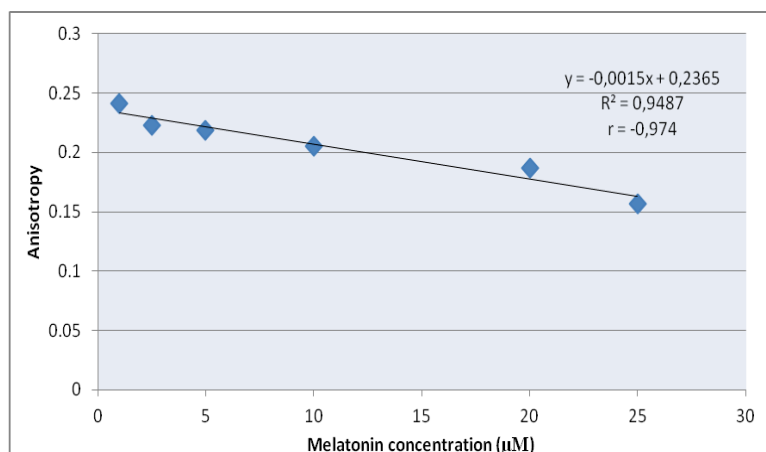
Figure 2

The dynamics of the fluorescence membrane anisotropy under the effect of the studied endogenous indols (melatonin, serotonin, tryptophan)

It can be noticed that serotonin and tryptophan do not modify significantly the experimental values of the membrane anisotropy. In addition, the smallest used concentrations (1 μM, 2,5 μM and 5 μM) of these indols determined a membrane rigidization of Jurkat cells, rendered by the increase of anisotropy levels (the decrease of membrane fluidity=rigidization).

By analyzing the registered results, a dose-dependent relationship of the isotropic effect of melatonin can be outlined. By increasing the concentration of melatonin, we acquired a progressive augmentation of the membrane fluidity.

The dose-response correlation (correlation between melatonin concentration and anisotropy) was statistically processed and is characterized by a Pearson correlation coefficient $r = -0.974$ (figure 3). We can assert that the increase of the melatonin concentration is consequently followed by a decrease of the membrane anisotropy, hence by a statistically significant increase of the membrane fluidity.

**Figure 3**

The influence of melatonin concentration on the fluorescence membrane anisotropy dynamics

Conclusions

Stimulating membrane fluidity is nowadays considered as an useful pharmacological method for enhancing the therapeutic effect of the chronic diseases pharmacotherapy. From this point of view, the results of the present study bring new and extremely important scientific information regarding the effects of some endogenous indolic compounds on neoplastic cells (lymphoblastoma) membrane fluidity.

Therefore, the obtained experimental results demonstrate for the first time, the stimulatory effect of melatonin, the pineal hormone, on membrane fluidity. Furthermore this effect is dose-dependent.

Melatonin proves to be an extremely efficient bio-indolic agent with potential pharmacotherapeutical approaches of chronic diseases, characterized by cellular membrane rigidization like neoplastic diseases.

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