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Measurement of fluorescence anisotropy as a parameter of membrane fluidity in patients with chronic kidney disease

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Introduction

Chronic kidney disease (CKD) represents diseases in presence of kidney damage or reduced levels of kidney function for more than 3 months [1]. In terminal stages, CKD results in loss of kidney function, dialysis, respectively kidney transplantation. We intended to investigate whether the early stages of CKD (specifically 2. - 3. stage) imply alterations in cell membrane properties. Anisotropy measurements with fluorescence probe are commonly used to measure fluidity of membranes. In biological membranes position of probe restricts the movement of phospholipids, probe oscillates with membrane phospholipids and so it can be used to measure the fluidity of membranes [2]. We chose red blood cells (RBC) membranes, because they represent the simplest usable model to study membrane fluidity since they avoid the contribution of other intracellular structures besides plasma membrane.

Material and methods

Twenty-one patients with stage 2.-3. of CKD and thirty-seven age- and gender-matched healthy volunteers with normal haematological and biochemical values were included in the study. The diagnosis of CKD was based on clinical and laboratory examinations as defined by the K/DOQI criteria [1].The Ethics Committee of Slovak Medical University approved the study and all participants gave their written informed consent. As a biological sample we used human blood, which was granted from Laboratory of Experimental and Clinical Biochemistry of Slovak Medical University in Bratislava. Blood was taken in tubes containing heparin, subsequently plasma, leukocytes and platelets were removed, and only red blood cells were used for this work. From erythrocyte mass we had isolated red blood cell membranes, we achieved "ghosts" using standard method Hanahan&Ekholm [3] modified in our laboratory to achieve a higher quality of the "ghosts". To measure fluorescence anisotropy we used DPH (1,6-diphenyl-1,3,5-hexatriene) probe. The stock solution of DPH (Serva, Germany), which was used during the measurements was prepared in acetone (Lachema, Czech Republic) and had concentration of $5x10^{-4}$ mol/L, was stored in the dark and refrigerated at 4°C. Before each measurement we prepared from the stock solution the dye working solution diluting it in TRIS (2 - amino - 2 - hydroxymethyl - propane - 1,3 - diol) (concentration of 20 mmol/L, pH 7.4) in a ratio of 1:250. After shaking (approximately 40 min) acetone was removed from the dye working solution. Measured sample (2000 mL) was composed of 1840 μL of TRIS (20 mmol/L, pH 7.4), 10 μL of "ghost" and 150 μL of the dye working solution. The final concentration of DPH in the sample was $1.5x10^{-7}$ mol/L. Stationary fluorescence anisotropy measurements in erythrocyte membranes, using DPH probes were made on a luminescent spectrophotometer Perkin-Elmer LS 45, single-channel method in the L-conformation, which is part of the software program FL WinLab. The sample was excited with linear polarized light and fluorescence was subsequently measured perpendicular to the direction of excitation beam. The experiment was based on time dependence measurements of stationary fluorescence anisotropy DPH probe $r_s(t)$ during incorporating the DPH probe into the erythrocytes membrane, up to 2000 seconds after its addition, with recording fluorescent intensity every 8.55 s. We used a constant excitatory wavelength of 360 nm (probe reaches the maximum absorption) and a constant emission wavelength length of 430 nm (maximum fluorescence). Measurements were conducted at room temperature (22 ± 2°C). In determining of erythrocyte membrane

fluidity from time evolution of the fluorescence anisotropy of probe DPH (at least 2000 s) we achieve the value of the stationary anisotropy by fitting with exponential curve in first order.

Results and discussion

We were interested in the fact whether CKD influences the membrane fluidity of red blood cells. To solve this problem we measured the value of DPH fluorescence anisotropy incorporated into the membrane after stabilization (approximately 2000 s). The process of DPH incorporation was fitted by exponential curve, do shown in Figure 1.

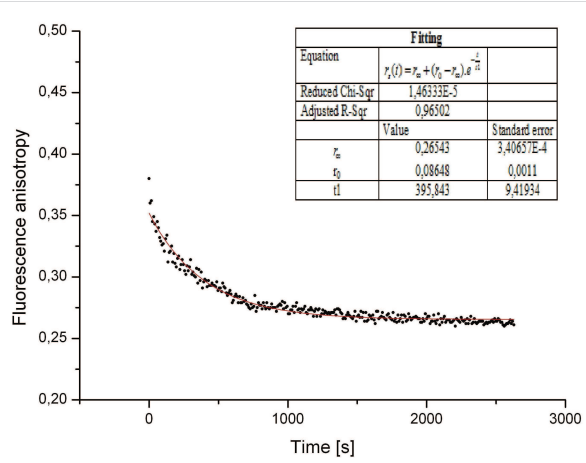


Figure 1: Typical time course of incorporation of probe DPH into the erythrocyte membrane in patient with chronic kidney disease with fitting curve, when r_∞ represent stationary fluorescence anisotropy, r_0 anisotropy in moment of adding DPH probe and t1 is a kinetic parameter of incorporating DPH probe.

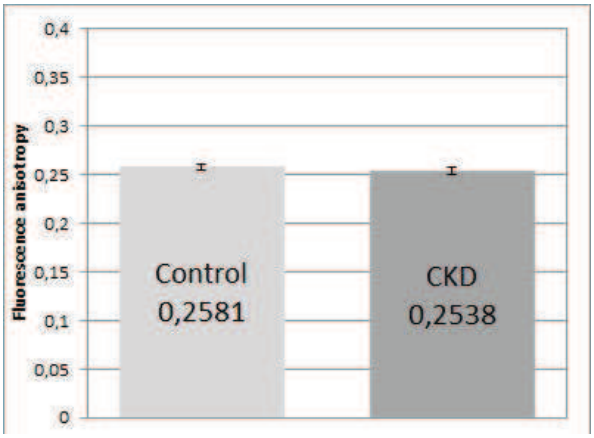


Figure 2: Comparison central value of fluorescence anisotropy by probe DPH in the column graph for control (n = 37) and CKD group (n = 21) with handed down by standard errors of mean (SEM)

The object of our interest is r_∞ parameter describing the fluorescence probe DPH at steady state, which gives us information on membrane fluidity. Graphic representation of mean values of fluorescence anisotropy using the DPH probe for CKD and control group in column format with standard errors of mean (SEM) gives us Figure 2. We assess that we found no significant difference of fluorescence anisotropy in patients with chronic kidney disease in 2. and 3. stage compared with the control group of healthy volunteers at the significance level $\alpha = 0.05$. It means that the level of motion of membrane phospholipids hydrophobic chains is not significantly different between CKD patients and healthy donors, at least in the early stages of CKD. It was previously shown that RBC from end stage CKD patients demonstrate reduction in membrane deformability, possibly as a result of increased RBC cellular calcium level [4]. Probably the changes in cell deformability occur only in the later stages of the disease.

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