

## Wroclaw University of Science and Technology



Poznań

June 27-29, 2016

# **MONITORING OF PLATELET MEMBRANE FLUIDITY DURING**

## **DIALYSIS OF SHEEP**

<u>K. Gałecka<sup>1</sup>, T. Walski<sup>1</sup>, J. Bujok<sup>2</sup>, K. Grzeszczuk-Kuć<sup>1</sup>, J. Detyna<sup>3</sup> and M. Komorowska<sup>1</sup></u>

<sup>1</sup> Department of Biomedical Engineering, Wrocław University of Science and Technology, Poland

<sup>2</sup> Department of Animal Physiology and Biostructure, Wrocław University of Environmental and Life Sciences, Poland

<sup>3</sup> Department of Mechanics, Materials Science and Engineering, Wrocław University of Science and Technology, Poland

## INTRODUTION

Platelet plasma membrane is thought to participate in mediating signal transduction processes. The changes of platelet membrane fluidity is one of the signs of the activation process or pathological states [1-8]. Among the methods to monitor molecular membrane organization is the spin probe method where paramagnetic molecules diffusing into a membrane and reflecting its rigidity states are used. Here we demonstrated a simple way

#### which provides information on alterations in the membrane fluidity of blood platelets.

The method was tested on sheep platelets upon haemodialysis (HD) procedures. In this procedure the interaction of blood with bioincompatible membranes of the dialyzer, which is a part of the extracorporeal circuit during hemodialysis, leads to activation of several cellular and non-cellular systems, results in a massive production and releases of reactive oxygen species, haemostasis disorders or impairment of blood elements [9-12]. It has been demonstrated that Near Infrared (NIR) radiation can produce a beneficial effects on blood cells, leukocytes and platelets under the influence of the NIR radiation reveal its protective impact: NIR stabilizes cellular membrane through increasing its resistance for destructive factors (mechanical) and oxidative stress [13-16]. In the present study NIR light was applied to examine its protective action during HD.

### **MATERIAL AND METHODS**

#### Haemodialysis procedure

The study was conducted on male Merino sheep. The animals undergone 2-step bilateral nephrectomy and were inserted permanent dual-lumen catheters into jugular veins. For each animal haemodialysis were repeated ten times as short daily HD sessions. Procedures were performed using Fresenius 4008S (Fresenius Medical Care, Germany) haemodialysis machines equipped with standard adult blood lines and F5 HPS polysulfone low-flux dialyzers (Fresenius MC, Germany). During each procedure blood was drawn prior to, after ca. 15 minutes and at the end of HD from the arterial and venous lines and were used as research material. In addition, blood of half sheep (five) flowing via dialyzer were exposed to near infrared (NIR) radiation (halogen lamp used with a transparency filter,  $\lambda = 780-1500$  nm).

### Platelet membrane fluidity test



In this method platelets in platelet-rich plasma (PRP) samples were studied to avoid washing procedures that activate cells. Literature studies demonstrate that surface activation of platelets on glass is associated with elevation of cytosolic calcium, protein kinase C translocation and talin redistribution. Surfaceactivated platelets respond to the action of agonists and mobilize ionized calcium [17]. Therefore the glass surface was employed as an activator by placing the PRP samples in glass capillaries. Surface-induced platelet activation and accompanied membrane changes were analyzed by electron paramagnetic resonance (EPR) measurements. The comparison of spectra of the 16doxylstearic acid labeled platelets in PRP, washed platelet suspension and albumin solution (Fig. 2) demonstrate that a ratio of intensities of the low-field,  $h_{+1}$ , and middle-field,  $h_0$ , lines of the spectra indicates the mobility of the spin probe incorporated into a lipid bilayer and reflects a platelet membrane lipid fluidity (Fig. 3). The EPR spectra of labeled platelets in PRP were recorded as a function of time (t = 0 when labeled)platelets were placed to the glass capillary) and a parameter of the membrane fluidity were calculated. Activation of platelets was associated with an increase of fluidity of the membrane to the equilibrium state which was reached after 10 min. contact with a glass surface (Fig. 4) that correspond to taking the fully spread form by platelets. The value of membrane fluidity in the equilibrium state was determined (EPRnas) and analyzed.

Fig. 1. HD session in sheep.



Fig. 5. Aggregation (ADP (a), collagen (b)) and membrane fluidity (EPR-nas, (c)) results throughout the period of experiment in control (K) and irradiated (NIR) group.



Fig. 2. The spectra of the 16-doxylstearic acid labeled platelets in PRP (blue), washed platelet suspension (red) and albumin solution (black).



Fig. 3. The spectrum of the 16-doxylstearic acid labeled platelets in PRP – determination of the parameter of the platelet membrane fluidity.



EPR spectra indicated the reduction of the membrane fluidity of platelets from blood exposed to NIR radiation in comparison to control samples. The results of experiments corresponded with better aggregability of irradiated platelets induced by both ADP and collagen (Fig. 6).

The results of EPR and aggregation methods revealed changes of parameters during single haemodialysis procedure and differences between control and irradiated samples taken before (B) and after (A) dialyzer (Fig. 7). The analysis of ADP-induced aggregation and membrane fluidity results in the course of dialysis demonstrated distinct patterns of control and irradiated group. The proposed method of monitoring of the platelet membrane fluidity was able to indicate a one-day gap in the HD sessions (between sixth and seventh dialysis session) (Fig. 8).





Fig. 4. The changes of the membrane fluidity parameter during platelet activation on glass as a function of contact time.

The calculated parameter of the platelet membrane fluidity demonstrated statistically significant differences:  $\succ$  in the course of dialysis; between control and irradiated group.  $\succ$  within single haemodialysis procedure;



REFERENCES

[1] M. Steiner and E.F. Lüscher, Biochemistry, 23(2), (1984)	[5] R.D. Sauerheber et al., J. Membr. Biol., 52(3), (1980) 201-	[8] C. Watala et al., Eur. J. Haematol., 61(5), (1998) 319-326	[14] M. Komorowska et al., 68, (2002) 93-100
247-252	219	[9] Holmes CJ., Artif Organs 19, (1995) 1126-35	[15] M. Shiraishi et. al., Luminescence, 14, (1999) 239–243
[2] A. Kantar et al., Mediators Inflamm., 1(2), (1992) 127-131	[6] S.J. Shatti, D.B. Cines and A.D Schreiber., J. Clin. Invest.,	[10] Babior BM., J Clin Invest, 73, (1984) 599-601	[16] L. Chludzińska et al., Blood Cells, Mol. Dis., 35, (2005) 74-
[3] I. Nathan et al., J. Biol. Chem., 254, (1979) 9822–9828	61(3), (1978) 582–589	[11] M. Olszewska, Ann. Acad. Med. Stetin, 50, (2004) 41-52	79
[4] S. Kitagawa, T. Shinohara and F. Kametani, J. Membr. Biol.	, [7] G.S. Zubenko et al., Psychopharmacology (Berl), 145(2),	[12] V. Sirolli et al., Int. J. Artif. Organs, 25, (2002) 529-537	[17] G.H. Rao, G. Escolar and J.G. White, Ann N Y Acad Sci.,
79(1), (1984) 97–102	(1999) 175–180	[13] M. Komorowska et al., 42, (1997) 379-386	714, (1994) 297-9