Acta Physica Universitatis Comenianae
Vol. XLVI–XLVII, (2005–2006) 91–94

Effect of Green Laser Light on Diabetes Mellitus Changed ATPase Activity in Erythrocytes

P. Kaššák, L. Šikurová

Division of Biomedical Physics, Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynská dolina, 842 48 Bratislava, Slovakia, e-mail: *sikurova@fmph.uniba.sk*

Abstract: Changes in the membrane bound enzyme activity may report about changes of processes and properties related to the cytoplasmic membrane of cells. Activity of the Na^+/K^+-ATPase has become objective of our investigation as a tool to evaluate changes of diabetic membranes in comparison to normal membranes of human erythrocytes after laser irradiation with Nd:YAG laser (532 nm) in fluence range 9.5 63.3 J.cm ^{2}. Energies of irradiation 3 20 joules and output power of the laser 30 mW classify this experiment as low–level laser therapy. Biostimulation of the enzyme, its activity as well as type-2 diabetes caused disorganization and alterations of biological membrane and enzyme properties are discussed.

1. Introduction

It has been convincingly demonstrated that diabetes mellitus (DM) causes metabolic disorganization and alterations of biological processes throughout the whole organism [1]. Abnormalities in function of receptors, ion transport and irregularities in function of cellular and subcellular membrane systems are important features of diabetes changed environment. In the last decade, low-level laser therapy (LLLT, 1 500 mW) has been subject of extensive investigation and, naturally, prospective applications of laser irradiation of various wavelengths appeared also in diabetes mellitus treatment. He-Ne laser irradiation is usefully applied to heal diabetic foot ulcers [2], cutaneous wounds [3, 4], diabetic macular edema [5] etc. But the mechanisms of diabetic cell response to laser irradiation are not clear yet.

Changes in the membrane bound enzyme activity may report about the changes of processes and properties related to cytoplasmic membrane of cell. In this study, activity of Na^+/K^+-ATPase has been objective of our investigation as a tool to evaluate changes of diabetic membranes in comparison to normal membranes of human erythrocytes. We compared activities of the Na^+/K^+-ATPase after irradiation by green laser of healthy donor samples with samples obtained from type-2 diabetes patients.

2. Materials and Methods

Membrane preparation

Fresh human blood was collected under the guidelines of the Helsinki Declaration for human research from anonymous adult healthy donors.

Blood was collected on citrate, centrifuged/washed by ice cold PBS (10 mM phosphate buffer, 150 mM NaCl, pH 7.4) to separate erythrocytes from plasma and other compounds. To obtain a suspension of isolated erythrocyte membranes (ghosts; 1 mg/ml) in 5 mM Tris-EDTA-HCl (pH 7.4) buffer, erythrocytes were hemolysed and centrifuged at 19621 g at least 6 times [6].

For unification, protein concentration in ghosts was estimated according to the standard method of Lowry et al. [7] using 3 % sodium dodecyl sulfate (SDS), and bovine serum albumin (BSA) as a standard. Ghosts were diluted to protein concentration 1 mg/ml.

Irradiation procedure

Second Harmonic Generation of Nd : YAG laser (Raise Electronics, Taiwan) with a constant light power of 30 mW was used as a source of green light (532 nm). A volume of 110 µl of each sample was irradiated for 100, 200, 300, 400, 500 and 680 seconds respectively, to get fluence (i.e. light energy per unit area of sample surface received by irradiated sample) of 9.5, 19.0, 28.4, 38.0, 47.5, and 63.3 J.cm ^{2} corresponding to energies of irradiation 3, 6, 9, 12, 15 and 20 Jouls. Non-irradiated samples were used as controls.

ATPase activity measurements

Activity of ATPases in erythrocyte membranes was determined in terms of liberation of inorganic phosphate during enzymatic ATP hydrolysis, and was expressed in nmol phosphate per mg of protein released during 30 min incubation (P_i). Calibration was based on KH_2PO_4 as a standard [8].

Statistical analysis

The data were analyzed using statistical software StatsDirect and p values < 0.05 were considered to be statistically significant. Results were expressed as mean ± standard error of mean (SEM) of 4 8 independent experiments. Data were examined for normal distribution by the Shapiro-Wilks W test. Statistical significance was evaluated by paired two tailed t test. Linear regression was used to prove the trend for the measurements.

3. Results

The effect of laser light (532 nm) on the activity of the Na^+/K^+-ATPase in the human erythrocyte membranes obtained from healthy donors and individuals diagnosed with type-2 diabetes was assessed by the quantity of inorganic phosphate (P_i) released in process of enzymatic hydrolysis of ATP. Our data (Fig. 1) showed an increase in the concentration of P_i produced by healthy individuals Na^+/K^+-ATPase in all irradiated samples as compared to non-irradiated ones. The tendency of the increase was proven statistically significant for all radiation energies used. The least-squares fit through the experimental points (concentration of P_i *vs* irradiation energy, Fig. 2) showed linearity ($R^2 = 0.96$, $p = 0.0001$) with a slope of 2.3 within the range of fluence values of 9.5 63.3 J.cm ^{2} corresponding to energies of irradiation 3 20 J.

Na^+/K^+-ATPase activity estimated on isolated erythrocytes obtained from individuals with diagnosed type-2 diabetes did not show any changes upon irradiation at all fluences (Fig. 1). Correlation coefficient was not significantly different from zero. The value of the

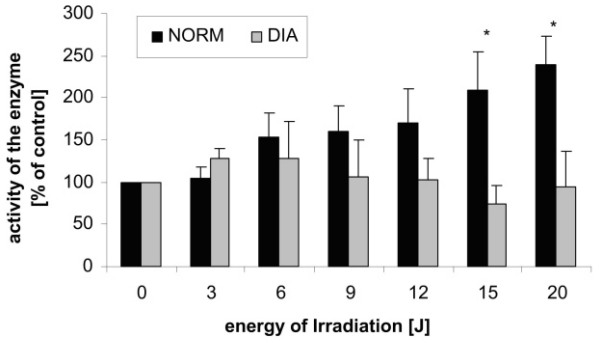

Fig. 1. Comparison of activities of Na^+/K^+-ATPase of red blood cells of healthy and diabetic individuals after the irradiation with Nd : YAG laser of various energies of irradiation. Data are presented as means ± SEM of the concentration of inorganic phosphate in percents compared to non irradiated control. * $p < 0.05$ *vs.* control.

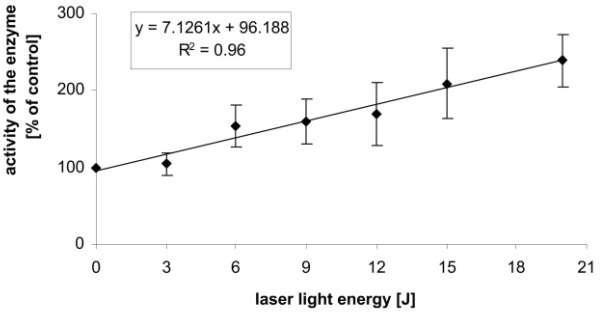

Fig. 2. Equation of trend line and coefficient of determination (R^2) for activity of Na^+/K^+-ATPase of red blood cells of healthy individuals after laser biostimulation. Results are reported as mean ± SEM of the concentration of inorganic phosphate (n = 8) in percents compared to non irradiated control.

P_i liberated during the enzymatic hydrolysis of the ATP was, with little differences, the same for all irradiated samples and non-irradiated control. Linear regression did not show significant trend in diabetic sample.

4. Discussion

In spite of successful applications of laser irradiation of various wavelengths on healing process of experimental animals with induced diabetes and patients with diagnosed diabetes, results obtained from experiments performed on cellular level vary from positive to no or negative effect of laser irradiation.

In general, increased glucose levels results in decrease in the viscosity of blood samples, increased hemolysis and osmotic fragility of erythrocytes [9] and higher erythrocyte transmembrane potential [10, 11]. High level of free glucose in the blood also intensifies the process of non-enzymatic glycation a reaction of reduced sugars with the primary amino groups of proteins [12].

It has been shown in diabetic tissues that there is a 2-fold increase in glycation of total membrane proteins comparing to control tissues [13].

Some positive results using green light irradiation at cellular level were shown by Vinck et al. [14], who reported increased proliferation of fibroblast growth under hyperglycemic conditions.

In our experiments, increasing activity of Na^+/K^+-ATPase after irradiation in control healthy donor samples may be explained as biostimulating effect of green light on the enzyme and is linearly proportional to energy of irradiation (9.5 – 63.3 J.cm ^{2}). Stimulation reached up to 238 % comparing to control (Fig. 1).

On the other hand nor stimulation neither inhibition of Na^+/K^+-ATPase activity has been visible after irradiation of diabetic samples. A decrease in activity [15] and particularly in the amount of Na^+/K^+-ATPase have also been observed in the diabetic myocardium [16]. This conservation of Na^+/K^+-ATPase in the diabetic erythrocytes seems to belong to a broader circle of adaptation changes on molecular level described before [1]. Changes in the membrane fluidity and the dynamics of cell membrane [17], removal or change in composition of membrane lipids may result in partial to total loss of enzymatic activity [18]. Thus, influencing of lipid properties can affect Na^+/K^+-APTase function as an enzyme strongly associated with cellular membrane. This may adversely affect protein function and structure which most probably results in lower response to gentle energy transfers from laser light irradiation.

Possibly longer or more intense irradiation would succeed in stimulation of the Na^+/K^+-ATPase as observed in non diabetic samples and will be objective of further investigation.

Acknowledgements

This study was supported by the VEGA 1/0253/03, APVT51-027404 and UK/346/2005.

References

[1] A. Ziegelhöffer: Gen. Physiol. Biophys. **19** (2000) 9.
[2] Z. Landau: Arch. Orthop. Trauma. Surg. **117** (1998) 156.
[3] K. R. Byrnes, L. Barna, V. M. Chenault et al.: Photomed Laser Surg. **22** (2004) 281.
[4] G. A. Maiya, P. Kumar, L. Rao: Photomed Laser Surg. **23** (2005) 187.
[5] F. Bandello, R. Pognuz, A. Polito et al.: Semin Ophthalmol. **18** (2003) 251.
[6] D. J. Hanahan, J. E. Ekholm: The preparation of red cell ghosts (Membranes). In: Methods in Enzymology 31. Fleischer S (ed), Academic Press, New York, (1974)168 177.
[7] O. H. Lowry, A. I. Rosebrough, N. J. Farr, R. Randall: J. Biol. Chem. **193** (1951) 265.
[8] L. Bonting, R. Canady: Am. J. Physiol. **207** (1964) 1005.
[9] B. Riquelme, P. Foresto, M. D'Arrigo et al.: J Biochem Biophys Methods. **62** (2005) 131.
[10] I. B. Zavodnik, A. Piasecka, K. Szosland, M. Bryszewska: Scand. J. Clin. Lab. Invest. **57** (1997) 59.
[11] I. Waczulíková, L. Šikurová, M. Bryszewska et al.: Bioelectrochemistry **52** (2000) 251.
[12] T. J. Lyons: Am. J. Cardiol. **71** (1993) 26B.
[13] E. Y. Zhang, P. W. Swaan: AAPS PharmSci. **1** (1999) E20.
[14] E. M. Vinck, B. J. Cagnie, M. J. Cornelissen et al.: Photomed. Laser Surg. **23** (2005) 167.
[15] G. N. Pierce, B. Ramjiwan, H. P. Meng: The Diabetic Heart. Ed. M. Nagano, N. S. Dhalla, New York: Raven Press 1991.
[16] K. Kjeldsen, H. Braendgaard, P. Sideniuset al.: Diabetes **36** (1987) 842.
[17] C. Watala: Diabetic medicine **10** (1993) 13.
[18] J. D. Robinson, M. S. Flashner: BBA **549** (1979) 145.