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EFFECT OF SELECTED SUBSTANCES WITH ANTIGLYCATIVE AND ANTIOXIDATIVE PROPERTIES ON ERYTHROCYTE DEFORMABILITY IN DIABETIC PATIENTS

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A b s t r a c t

The aim of this study was to investigate aminoguanidine, pyridoxylidenaminoguanidine and pyridoxal in terms of their protective influence on erythrocyte elasticity. In seven non-diabetic subjects and 16 diabetic patients with insulin-dependent diabetes mellitus, the elasticity of erythrocytes was assessed on the basis of their deformability by a filtration method with centrifugation. Other haematological variables, including erythrocyte and reticulocyte counts, haematocrit values, haemoglobin concentrations and the mean cell volume, were determined. All substances showed a mild protective effect on erythrocyte elasticity in both the healthy subjects and the diabetic patients. A significant improvement in elasticity was obtained only by pyridoxal ($P < 0.01$) in the patients with diabetes mellitus. The haematological values found were in the range of physiological values in both groups.

K e y w o r d s

Erythrocyte elasticity, Membrane active substances, Diabetes mellitus

A b b r e v i a t i o n s u s e d

AG, aminoguanidine; PAG, pyridoxylidenaminoguanidine; P, pyridoxal; IDDM, insulin-dependent diabetes mellitus

INTRODUCTION

The elasticity of the erythrocyte membrane enables the erythrocyte to adapt its shape to the lumen of capillaries, which is a prerequisite for blood perfusion and, consequently, blood supply to tissues.

A reduced elasticity of the erythrocyte membrane is found in some haematological, malignant and metabolic diseases (diabetes mellitus), in myocardial infarction and under stressful conditions or during hard exercise (*1*). On the other hand, mild physical activity and also substances with antioxidant effects improve erythrocyte deformability. Recently, substances that act on the membrane and thus positively influence its elasticity and improve erythrocyte deformability have been studied.

The aim of the study was to monitor the effect of selected substances, i.e., aminoguanidine, pyridoxylidenaminoguanidine (newly synthesised adduct of aminoguanidine with pyridoxal) and pyridoxal on erythrocyte deformability in patients with insulin-dependent diabetes mellitus and in non-diabetics.

MATERIALS AND METHODS

Two groups of students were examined. The first consisted of 16 IDDM patients aged 21 to 31 years. For an average of 14 years, they had been treated with insulin at an average concentration of 11.7 mmol.l^{-1} for glycaemia and with 9.04 % glycated haemoglobin. The second group included seven healthy subjects, non-diabetics, aged 20 to 23 years.

Samples of venous blood for haematological analysis and erythrocyte deformability determination were taken into sterile syringes with an anticoagulant; they were processed within 4 h of venepuncture. Erythrocyte elasticity was determined, according to the degree of erythrocyte deformability, by a filtration method with subsequent centrifugation. Deformability was calculated as the percentage of filtered erythrocytes from the total erythrocyte count. In addition to the erythrocyte count, other examinations included the haematocrit value, mean cell volume, haemoglobin concentration and reticulocyte count. The influence of AG, PAG, and P on erythrocyte deformability was tested for each assay. Three diluted blood samples with each tested substance at a concentration of $6.25.10^{-4}$ mol.l^{-1} and a diluted blood sample, which was substance–free, were incubated at 37°C for 1 h. All diluted blood samples were examined separately. The effect of substances on erythrocyte deformability was derived from a comparison of the deformability of samples incubated with AG, PAG and P with the deformability of the control (substance–free) sample.

Experimental data were statistically evaluated by the non-parametric Kolmogorov-Smirnov test, using a Statgraphic 4.0 programme.

This study was approved by the Ethic Committee of the School of Medicine, Comenius University, Bratislava, and carried out in accordance with the Declaration of Helsinki.

RESULTS

The average value of erythrocyte deformability after incubation at 37°C for 1 h was 66.3 ± 6.9 % in the healthy subjects. After addition of either AG or PAG, this value increased to 69.0 ± 6.7 % and 75.7 ± 8.0 %, respectively. After addition of P, erythrocyte deformability increased to 74.7 ± 8.1 %, as against the control sample (*Fig. 1*). In the diabetic patients, the average value of erythrocyte deformability after the same incubation was 69.0 ± 3.65 %; the effects of AG, PAG and P resulted in values that increased to 72.2 ± 4.23 %, 73.2 ± 3.51 % and 77.5 ± 3.2 %, respectively. The last value was statistically significant ($P < 0.01$; *Fig. 2*).

The other haematological variables for healthy subjects and diabetic patients were as follows: erythrocyte counts, $4.7 \pm 0.4.10^{12}.l^{-1}$ and $5.3 \pm 0.5.10^{12}.l^{-1}$; mean cell volume, 82.7 ± 6.4 10^{-15} l and 87.8 ± 3.5 10^{-15} l; haematocrit levels, blood haemoglobin concentrations and reticulocyte counts were in the range of physiological values. In diabetic patients, biochemical variables included an average glucose value of 11.7 mmol.l^{-1} and a glycated haemoglobin concentration (HbA_1c) of 9.04 %; in the healthy subjects, these variables were in the range of physiological values.

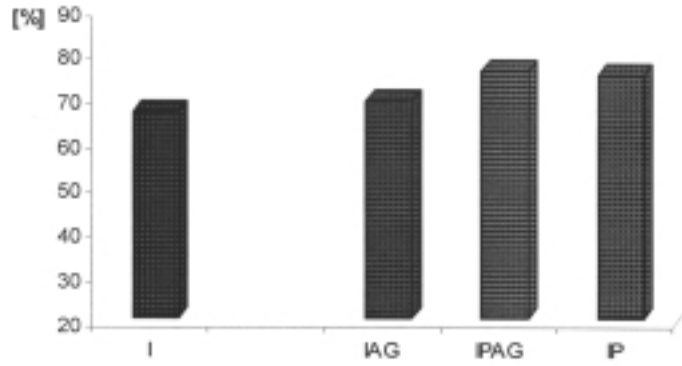

Fig. 1

Effects of AG, PAG and P on erythrocyte elasticity in non-diabetics. I, incubated sample; IAG, sample incubated with aminoguanidine; IPAG, sample incubated with adduct AG and P; IP, sample incubated with pyridoxal.

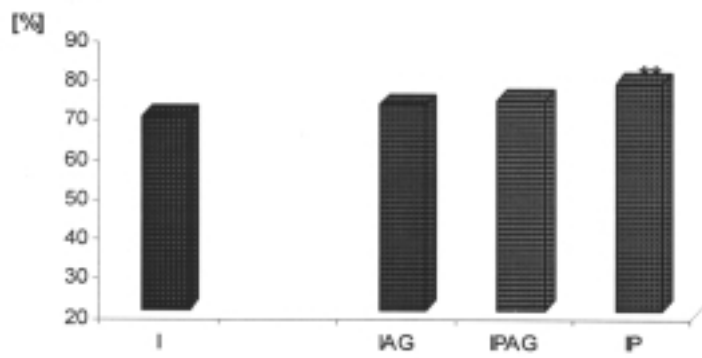

Fig. 2

Effects of AG, PAG and P on erythrocyte elasticity in diabetics. I, incubated sample; IAG, sample incubated with aminoguanidine; IPAG, sample incubated with adduct AG and P; IP, sample incubated with pyridoxal.
**, $P < 0.01$ (Kolmogorov-Smirnov test, Statgraphics 4.0 programme).

DISCUSSION

The content of oxygen in blood, its binding to and release from haemoglobin and the subsequent tissue perfusion are physiological factors involved in oxygen supply to the organism. Erythrocytes have the crucial role in these processes because their metabolism (biosynthesis of 2,3-DPG, ATP) is related to the ability of binding, transporting and releasing oxygen. Their adaptation to the narrow lumen of capillaries (*2*) depends on the degree of erythrocyte membrane elasticity, which is an inevitable prerequisite for the movement of erythrocytes through the microcirculatory net. Erythrocytes with a lower degree of deformability assume spherical shape and lose their ability to pass through narrow capillaries. As a consequence, they aggregate and accumulate in the terminal parts of arterioles, or capillaries, which causes embolism and subsequent ischaemia of the tissue involved. The shortage of oxygen in tissue is directly associated with free oxygen radical production and this has a negative influence on erythrocyte deformability and further development of ischaemia (*3, 4*).

The assessment of erythrocyte deformability provides new possibilities for evaluation of various pathological processes and situations in patients with impaired microcirculation (*5*). This parameter helps to specify diagnostic and therapeutic procedures in clinical practice. Changes in erythrocyte elastic properties can cause changes in perfusion through microcirculation. A decrease in erythrocyte elasticity due to haemoglobin glycation and an alteration of membrane proteins have been reported, for instance, in diabetes mellitus (*6),* ischaemic heart disease, hypertension (*7,8*) or obesity.

The influence of medication on erythrocyte deformability has not been studied sufficiently. Opioids are known to have a negative influence on erythrocyte deformability whereas xantine products and antioxidants have a positive effect. Some other substances with a potentially positive effect, such as derivatives of vitamin K, have also been tested.

In this paper, we focused on the effects of AG, PAG and P. Their anti-oxidant activity and inhibitory action in the pathogenesis of chronic diabetic complications have been described (*9, 10, 11, 12*). All substances tested here demonstrated a mildly protective effect on erythrocyte elasticity. AG improved the filtration ability of erythrocytes by 4 %, PAG by 11 % and pyridoxal by 13 % in the healthy subjects. In the diabetic patients, the erythrocyte filtration ability was improved by 7 % and 9 % due to the effects of AG and PAG, respectively.

In conclusion, all tested substances demonstrated a mild protective effect on erythrocyte elasticity in both groups examined. In the patients with diabetes mellitus, a significant increase by 15 % ($P < 0.01$) in the filtration ability of erythrocytes and, therefore, an effect on elasticity were achieved by the influence of pyridoxal.

A c k n o w l e d g e m e n t

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VPLYV VYBRANÝCH LÁTOK S ANTIGLYKAČNÝMI A ANTIOXIDAČNÝMI VLASTNOSŤAMI NA DEFORMABILITU ERYTROCYTOV U DIABETIKOV

S o u h r n

V práci sa sledoval účinok aminoguanidínu, pyridoxylidénaminoguanidínu a pyridoxalu na elasticitu erytrocytov u 7 diabetických a 16 nediabetických pacientov filtračnou metódou. Všetky testované látky mali mierny pozitívny účinok na elasticitu erytrocytov, avšak signifikantné zlepšenie elastických vlastností sa zaznamenalo len v skupine s pyridoxalom ($p < 0,01$) u pacientov s diabetes mellitus.

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