EXPERIMENTAL STUDY

The effect of selected membrane active substances on erythrocyte deformability

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Abstract

Aminoguanidine improved the erythrocyte filterability by 4 %, pyridoxyliden-aminoguanidine by 11 % and pyridoxal by 13 % in healthy subjects. In diabetic patients the aminoguanidine effect on erythrocyte filterability was improved by 7 %, PAG effect by 9 % and pyridoxal effect by 15 % in comparison to the control group. The other investigated haematological variables in both groups were within the range of the physiological standard.
All of the tested substances demonstrated a mild protective influence on erythrocyte elasticity both in healthy subjects and diabetic patients. Significant elasticity improvement was obtained only by pyridoxal ($p<0.01$) in patients with diabetes mellitus. ***(Fig. 4, Ref. 18.)***
Key words: elasticity of erythrocyte membrane, membranal active substances, diabetes mellitus, erythrocyte deformability.

The erythrocyte deformability is an important physiological property of erythrocytes mainly in microcirculation. It enables them to change their shape and adjust it to the lumen of the nutritive capillary bed and then to secure their primary function – oxygen transport to tissues. It was found out that in some organ diseases of the cardiovascular system (1–6), diabetes mellitus (7) and sepsis (8), the erythrocyte membrane elasticity and microcirculation impair, thus deteriorating the pathophysiological course of the disease. At present substances with positive effect on erythrocyte membrane fluidity and their deformability are being surveyed in order to be able to affect favourably the clinical manifestations of disease as well as the therapeutical results.

The aim of the work was to monitor membranal active substances with antioxidative properties: aminoguanidine (AG), pyridoxyliden-aminoguanidine (PAG) and pyridoxal (P) with presumptive protective influence on erythrocyte deformability in patients with insulin dependent diabetes mellitus (IDDM) and in non-diabetics.

Materials and methods

Two groups of students were examined. The first consisted of 7 healthy subjects, non-diabetics aged 20–23. The second group involved 16 diabetic patients aged 21–31 years with well compensated diabetes mellitus, who have been treated with insulin medication for an average of 14 years. The venous blood samples for haematologic analysis and erythrocyte deformability determination were taken into sterile syringes with anticoagulant in both groups and were treated no later than four hours after the venepuncture.

Erythrocyte deformability was determinated by means of the filtration method connected with centrifugation. Erythrocyte deformability was calculated as a percentage of filtered erythrocytes from the total erythrocyte count. Other haematologic variables – the erythrocyte count and haematocrit value were measured by using the semiautomated cell counter Picoscale 5, and haemoglobin concetration by the cyanmethaemoglobin method respectively.

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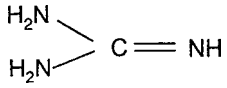
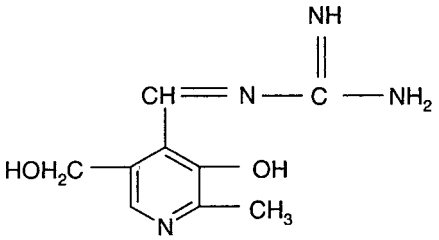
Acknowledgement. We thank Rusnakova J, Varinyova J, Frastacka O, Opalena D, Chnapek S for their technical cooperation.

Observed substances

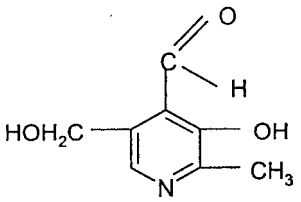
1) Aminoguanidine



2) Pyridoxyliden-aminoguanidine — new synthetized adduct of aminogunidine with pyridoxal



3) Pyridoxal



The concentration of tested substances with the reactive mixture of all blood samples was $6.25.10^{-4}$ mol.L^{-1}. Blood suspension with diluting solution and tested substances were incubated at 37 °C for 1 hour. After the incubation all blood samples were centrifuged and filtered to calculate erythrocyte deformability. The influence of tested substances on erythrocyte deformability was compared with erythrocyte deformability in the incubated control sample without tested substances. The experimental data were statistically evaluated by non-parametric Kolmogorov–Smirnov test using Statgraphics 4.0 programme.

Results

In the group of non-diabetic subjects, the average value of erythrocyte deformability calculated as a percentage of filtered erythrocytes after aminoguanidine effect was increased from 66.3±6.9 % to 69.0±6.7 %. After the addition of PAG it was increased to the value of 73.4±8.0 % and after pyridoxal effect to 74.7±8.1 % (Fig. 1).

In the group of diabetic patients, the average value of glycaemia was 11.7 mmol.L^{-1} and the percentage of glycated haemoglobin was 9.04 %. The average value of erythrocyte deformability after the addition of aminoguanidine was increased from 67.3±3.65 % to 72.2±4.23 % and after PAG effect to 73.4±3.51 %. After the addition of pyridoxal a significant improvement of erythrocyte deformability to 77.5±3.2 % ($p<0.01$) (Fig. 2) was found in the group of diabetic patients. The erythrocyte count in the group of non-diabetics subjects was $4.7\pm0.4.10^{12}$.L^{-1}, and in the group of diabetic patients it was $5.3\pm0.5.10^{12}$.L^{-1}, the haematocrit value, and blood haemoglobin concentration were in the ranges of physiological values and there were no significant differences between both groups (Fig. 3, 4).

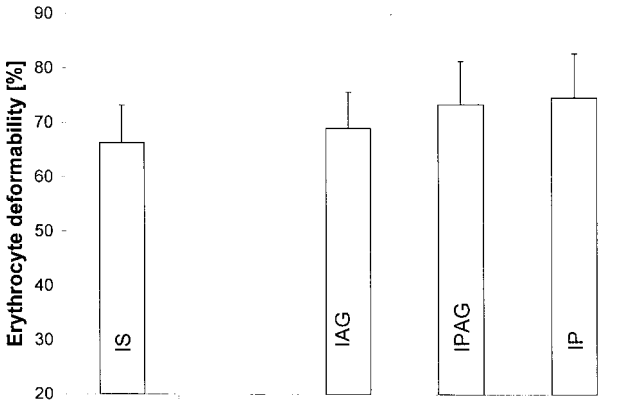


Fig. 1. Effects of AG, PAG and P on erythrocyte deformability in non-diabetics subjects. IS — incubated sample, IAG — with aminoguanidine, IPAG — pyridoxyliden-aminoguanidine, IP — pyridoxal.

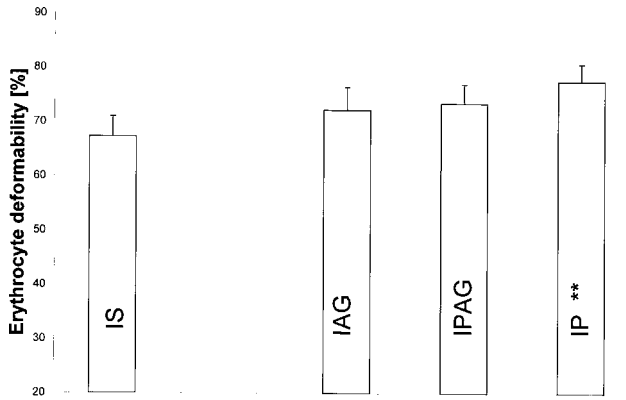


Fig. 2. Effects of AG, PAG and P on erythrocyte deformability in diabetics patients. IS — incubated sample, IAG — with aminoguanidine, IPAG — pyridoxyliden-aminoguanidine, IP — pyridoxal. ** $p<0.01$ Kolmogorov–Smirnov test, Statgraphics 4.0 programme.

Discussion

The erythrocyte deformability is a very important physico-mechanical factor influencing the transport of oxygen to tissues. When passing through the microcirculatory net the erythrocytes being bigger than the capillary diameter are able to change their shape and adapt to the narrow lumen of capillaries. The ability of erythrocytes to deform their shape depends on the degree of erythrocyte membrane elasticity. Erythrocytes with lower deformability gain spherical shapes, they cumulate in the termi-

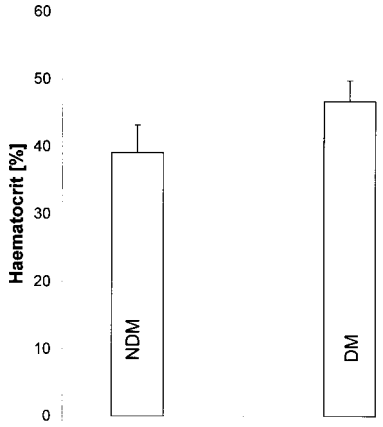


Fig. 3. Haematocrit value in non-diabetic subjects (NDM) and patients with diabetes mellitus (DM).

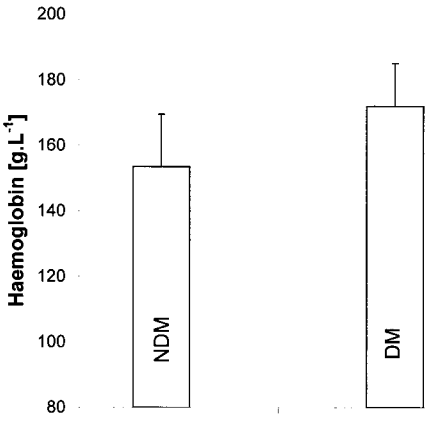


Fig. 4. Haemoglobin concentration in non-diabetic subjects (NDM) and patients with diabetes mellitus (DM).

nal parts of arterioles or capillaries, and the mechanic embolising is caused with subsequent tissue ischaemization and hypoxaemization. These pathological processes within the tissue deteriorate patient's clinical status and the basic disease is complicated. They might be also the cause of various kinds of angiopathy.

With regard to the fact that changes of erythrocyte membrane elasticity in various disease are found and serious clinical complications are caused, the determination of erythrocyte deformability is very important and it provides new possibilities for the assessment of diagnostic and therapeutic procedures in patients with impaired microcirculation.

Recently, the substances that act on the erythrocyte membrane and thus positively influence its elasticity have been studied. Positive effect of antioxidant substances is known. Application of alpha-tocopherol immediately after thermal skin injury improves erythrocyte deformability and protects erythrocytes against membrane peroxidative damage and rheological impairments (9, 10). After prolonged application of xantine preparatives the increased erythrocyte deformability was observed as well as beneficial effect of ischemic attacks occurrence (11). The positive effect of pyrimido-pyrimidine derivatives on erythrocyte deformability as well as endothelin-1 through the activation of protein kinase C was found. Also the negative effect of opioids on erythrocyte deformability is known (12, 13).

In our work the selective substances aminoguanidine, pyridoxyliden-aminoguanidine and pyridoxal with a presumptive positive effect on erythrocyte membrane were studied. All tested substances demonstrated a mild protective effect on erythrocyte membrane elasticity. Aminoguanidine improved the erythrocyte filterability by 4 %, PAG by 11 % and pyridoxal by 13 % in healthy subjects. In diabetic patients due to the aminoguanidine effect, erythrocyte deformability was improved by 7 %, PAG improved the deformability by 9 %. The significant positive influence on filterability by 15 % ($p<0.01$) and thus on erythrocyte elasticity was achieved after pyridoxal application in patients with diabetes mellitus. In these substances the antioxidative activity and inhibitive influence in pathogenesis of chronic diabetic complications were found (14, 15, 16). A new potential drug resorcylidene aminoguanidine affects lipid-protein interaction in erythrocyte membranes resulting in membrane lipid bilayer fluidization and leading to the restoration of natural physiological membrane dynamic parameters in erythrocytes from diabetic patients (17, 18).

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Received January 15, 2003.
Accepted February 7, 2003.