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EFFECTS OF NITRIC OXIDE AND PROSTACYCLIN
ON DEFORMABILITY AND AGGREGABILITY OF RED BLOOD
CELLS OF RATS *EX VIVO* AND *IN VITRO*Chair of Pharmacology, Medical College of Jagiellonian University, 16 Grzegórzecka, Cracow,
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Although many diseases of the heart and circulatory system have been linked with insufficient deformability and increased aggregability of red blood cells, there are only a few drugs which can modulate these biological functions of erythrocytes. Here, we show evidences that iloprost, stable prostacyclin analogue and SIN-1, active metabolite of molsidomine which spontaneously releases NO, may be sufficient pharmacological tools for modulating red blood cell deformability and aggregability. Deformability of red blood cells was measured by shear stress laser diffractometer (Rheodyn SSD) and expressed in percent of red blood cell deformability index (DI). MA-1 (Myrenne) erythrocyte aggregometer was used for photometric measurements of aggregability in arbitrary units (MEA) of mean extent of aggregation. Experiments were carried out on rats *ex vivo* and *in vitro* using whole rat blood or isolated erythrocytes. *Ex vivo* SIN-1 (infusion 2 mg/kg/min i.v.) and iloprost (bolus injection 10 µg/kg i.v.) significantly improved erythrocyte deformability and aggregability at 5—15 min after administration. L-NAME (10 mg/kg i.v.) — inhibitor of nitric oxide synthase, and aspirin (1 mg/kg i.v.) caused worsening of deformability of erythrocytes in experiments *ex vivo*. Studies *in vitro* also revealed improvement of red blood cell deformability and aggregability by SIN-1 (3 µM, 15 min incubation at 22°C) or iloprost (1 µM, 15 min incubation at 22°C) and this phenomenon appeared not only in whole blood but also in isolated red cells. It is concluded that NO- and prostacyclin-induced improvement of red blood cell deformability and aggregability results from direct action of these compounds on erythrocytes. NO-donors and iloprost could be useful in the treatment of disorders of blood fluidity.

Key words: *red blood cell deformability, red blood cell aggregation, nitric oxide (NO), prostacyclin, aspirin, L-NAME.*

INTRODUCTION

Flow, deformability and aggregability of blood cells are three main components of blood rheology. In large vessels, a basic component is the flow, because viscosity of blood depends on red cell concentration and plasma viscosity. In microcirculation where cells must deform to pass through capillaries, deformability and aggregation of individual cells are the major

determinant of resistance to flow. The ability to be deformed is crucial to the red cell for performing its function of oxygen delivery and it is also determinant of the cell survival time in the circulation (1). Altered deformability is observed either in hereditary disorders (spherocytosis, elliptocytosis, mutations of haemoglobin), or it is caused by environmental factors, such as oxidative stress and decreased antioxidant status (2). Changes in blood rheologic properties were observed in essential arterial hypertension (3,4), diabetes mellitus (5,6), atherosclerosis, red cell aging (7) and in clinical and experimental sepsis (8, 9, 10). However, there are only a few drugs which can modulate rheological functions of erythrocytes (11, 12). We found that prostacyclin and nitric oxide (NO) would play an important role in regulation of red blood cell deformability (13) when released from various endogenous sources such as leukocytes or vascular endothelium. Here, we study whether nitric oxide and prostacyclin may be sufficient pharmacological tools for regulation of deformability and aggregability of red blood cells during exogenous application.

MATERIALS AND METHODS

Procedures ex vivo

Male Wistar rats were anaesthetized with thiopental (90 mg/kg i.p.) and heparinized (800 U/kg i.v.). Blood samples (0.5 ml) were taken from the right carotid artery before (control sample) and after administration of the following compounds into left femoral vein: 1) SIN-1 infused at a dose of 2 mg/kg/min for 10 min and samples taken after 5 and 10 min (in some experiments 10 min infusion of SIN-1 was preceded with bolus injection of L-NAME at a dose 10 mg/kg), 2) iloprost in bolus injection at a dose of 10 µg/kg and sample taken after 15 min, 3) N^G-nitro-L-arginine methyl ester (L-NAME) in bolus injection at a dose of 10 mg/kg and samples taken after 15, 30 and 45 min, 4) aspirin in bolus injection at a dose of 1 mg/kg and samples taken after 15, 30 and 45 min.

Procedures in vitro

Male Wistar rats were anaesthetized with thiopental (90 mg/kg i.p.). The blood (8 ml) was collected from the right carotid artery into heparin (10 U/ml) solution. Erythrocytes were isolated using the method described by Jubelin and Gierman (14). Red blood cells were separated from white blood cells on a Ficoll packed column, then washed three times in PBS solution, enriched in albumin and glucose. Cells were suspended in solution of 500 PBS with 3% dextran for 30 min and again rinsed to remove dextran. Finally, cells were resuspended in PBS solution. Haematocrit was adjusted to the value of whole blood haematocrit. Neither white blood cells nor platelets were seen in prepared in this way suspension of erythrocytes during microscopic examination. Samples of whole blood or suspension of erythrocytes in PBS were incubated at 22°C with SIN-1 (3 µM) or iloprost (1 µM) or placebo (saline) for 15 min.

Red blood cell deformability

Erythrocyte deformability was measured using the shear stress laser diffractometer (Rheodyn SSD). This kind of instrument measures ellipsoidal elongation of red blood cells in response to defined shear stresses, determined by rotation rate. Samples of blood (30 µl) were suspended in

2 ml of dextran solution (MW 60 000, osmolarity 300 mOsm, pH = 7.4, viscosity 24 mPa). Red blood cell deformability index (%) was defined as $DI = 100(L-W)/(L+W)$, where L and W were the means of length and width of elongated red blood cells, respectively, which had been electronically calculated from the diode output of the sensor head of the instrument. For all experiments deformability indices of red blood cells were registered at the shear stress of 60 Pa.

Aggregability of red blood cells

Aggregability was measured using the aggregometer type MA1 (Myrenne gmbh) for erythrocytes with incorporated photometer working in the infrared range. In such an instrument assayed samples (20 μ l of whole blood or suspension of isolated erythrocytes) are applied into the chamber and submitted to a high shear rate (600 1/s) and to a low shear rate of 3 1/s in succession for 10 sec. After shear stop the mean extent of aggregation (MEA) is automatically measured, calculated and displayed in arbitrary units of MEA as a four-digit-number.

Statistical analysis

Results were expressed as arithmetical means \pm SD of *n* numbers of experiments and analyzed by Student's *t* test for paired means to determine the significance of the response. A „*p*” values of less than 0.05 were considered as statistically significant.

RESULTS

In experiments *ex vivo* SIN-1 at a dose of 2 mg/kg/min i.v. significantly ($p < 0,01$) increased deformability index (DI) of erythrocytes both after 5 min of infusion ($44,8\% \pm 1,9$; $n = 6$) and after 10 min of infusion ($45,0\% \pm 2,2$; $n = 6$) as compared with control ($43,6\% \pm 2,1$; $n = 6$) (Fig. 1). At the same dose SIN-1 after 5 and 10 min of infusion also significantly decreased aggregability of erythrocytes as measured during low shearing for 5 sec ($3,4 \pm 1,6$; $n = 6$; $p < 0,01$ and $3,4 \pm 1,4$; $n = 6$; $p < 0,05$, respectively vs control $4,0 \pm 1,5$; $n = 6$) (Fig. 2). Iloprost 15 min after injection caused increase in erythrocytes DI ($46,4\% \pm 2,0$; $n = 6$ vs control $43,4\% \pm 2,3$, $n = 6$; $p < 0,05$) (Fig. 1) and decreased erythrocyte aggregability ($3,8 \pm 0,8$; $n = 6$ vs control $4,6 \pm 1,1$; $n = 6$; $p < 0,01$) (Fig. 2). Inhibitor of NO-synthase, L-NAME, decreased red blood cell DI to $42,0\% \pm 3,4$ ($n = 9$; $p < 0,01$) at 15 min, to $38,3\% \pm 0,2$ ($n = 4$; $p < 0,05$) at 30 min and to $38\% \pm 0,5$ ($n = 4$; $p < 0,05$) at 45 min after injection, as compared to control experiments ($43,7\% \pm 2,9$; $n = 9$) (Fig. 3). L-NAME-induced impairment of red blood cell deformability was counteracted by SIN-1 at a dose of 2 mg/kg/min infused i.v. for 10 min (Fig. 4). Aspirin decreased DI at 15, 30 and 45 min from injection to $41,6\% \pm 3,4$ ($n = 6$; $p < 0,05$), $41,0\% \pm 2,5$ ($n = 6$; $p < 0,05$) and to $41,6\% \pm 3,0$ ($n = 6$; $p < 0,05$), as compared with control ($43,7\% \pm 3,0$; $n = 6$), respectively (Fig. 3).

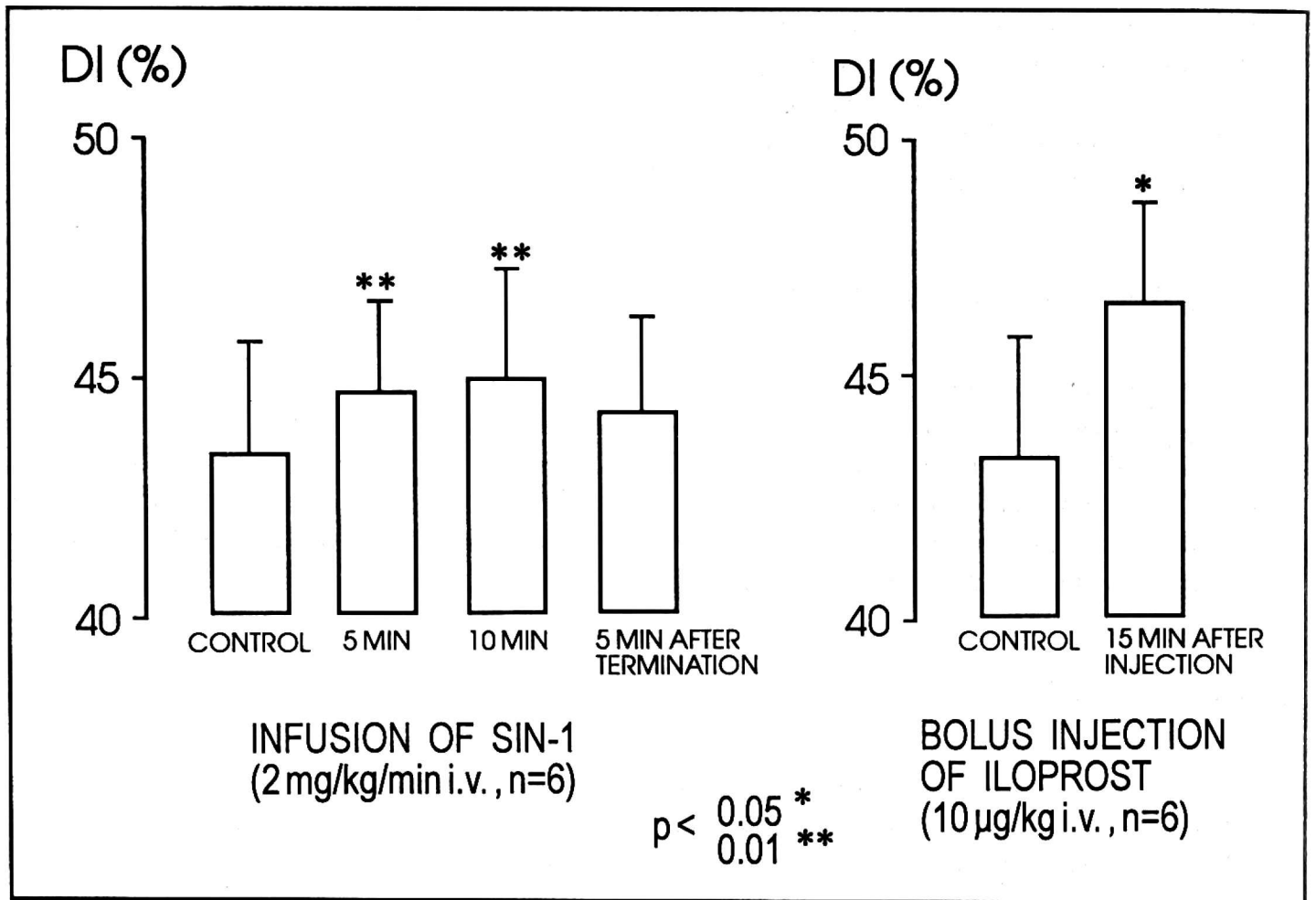


Fig. 1. The effect of SIN-1 or iloprost on red blood cell deformability *ex vivo*.

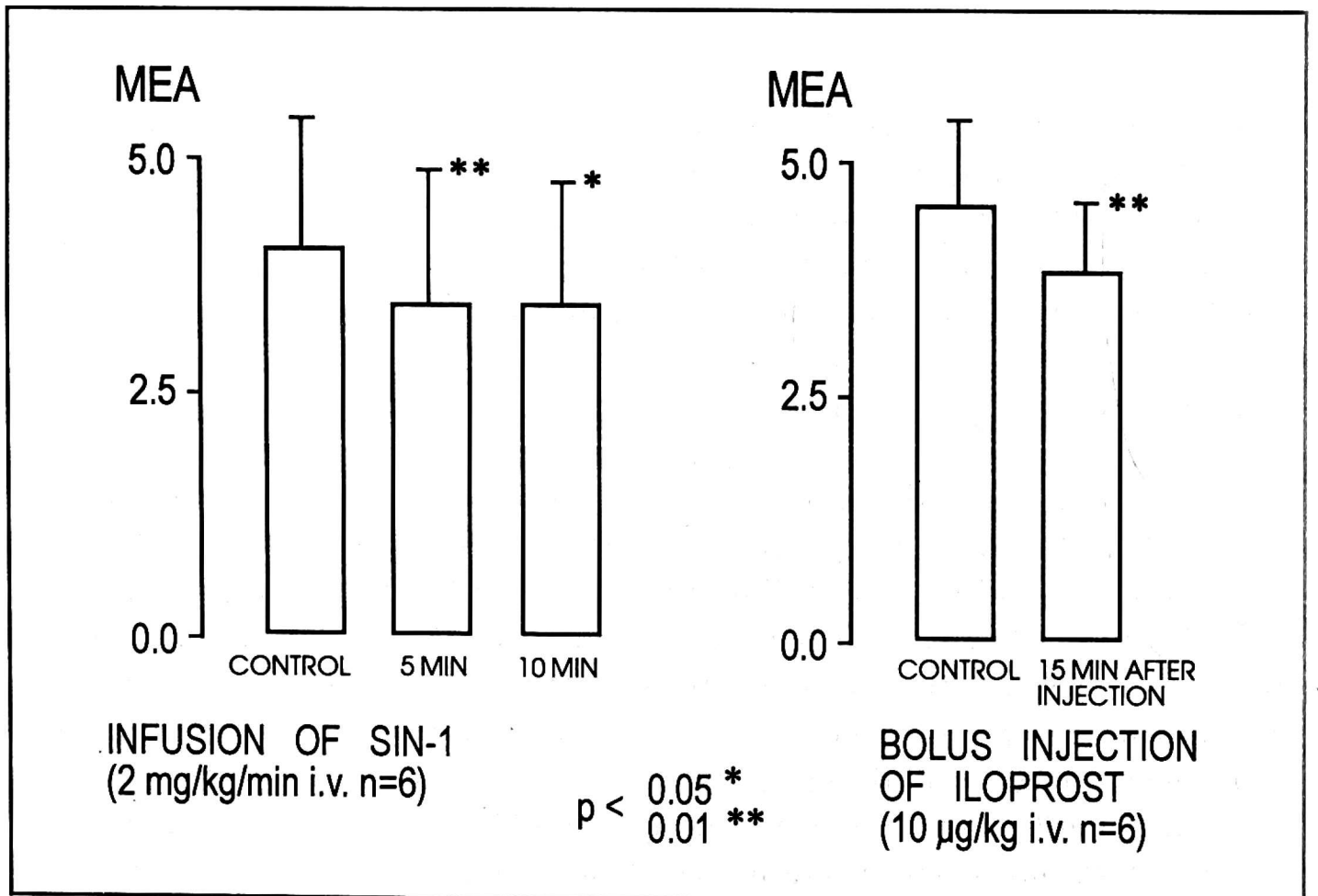


Fig. 2. The effect of SIN-1 or iloprost on red blood cell aggregability *ex vivo*.

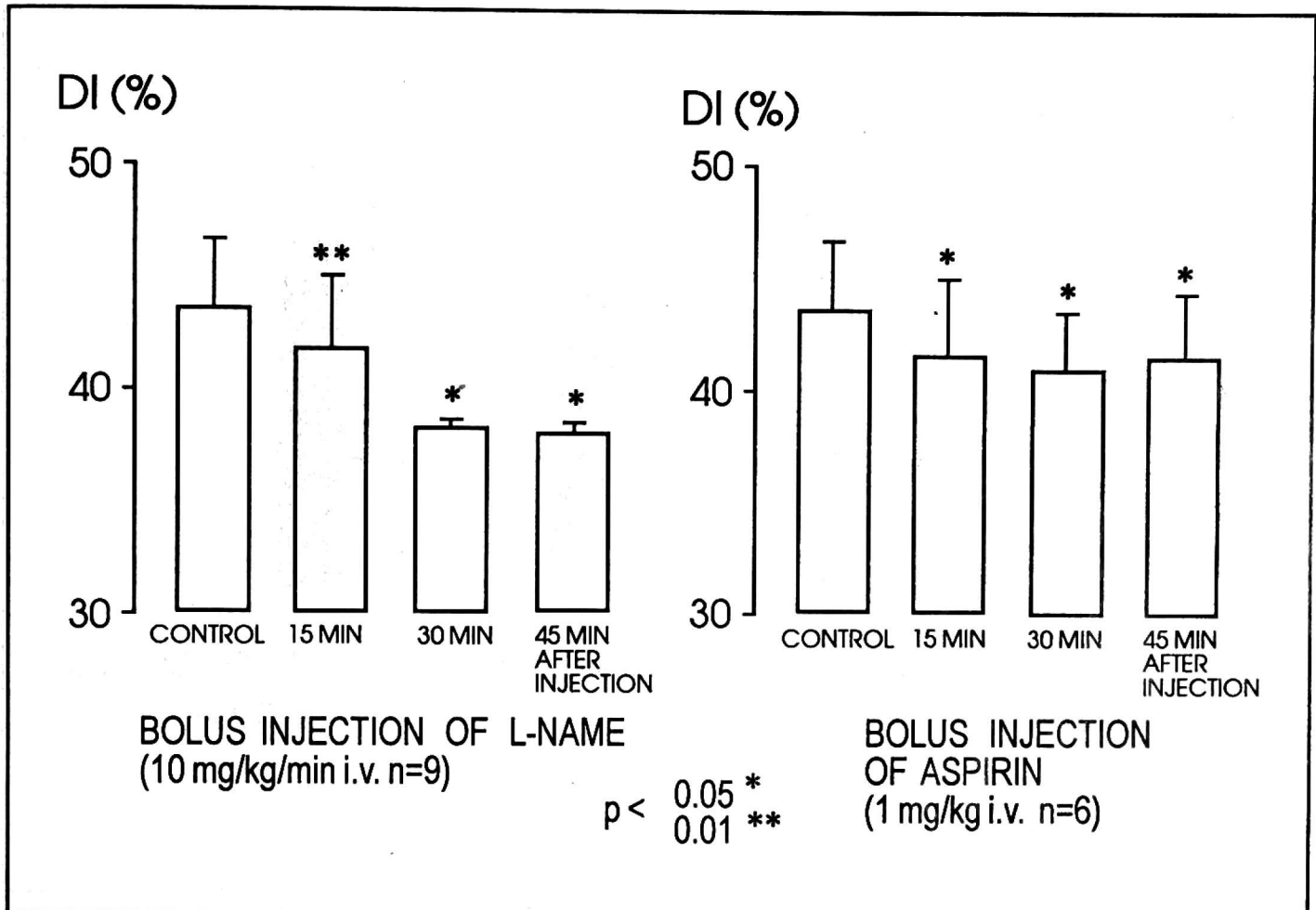


Fig. 3. The effect of L-NAME or aspirin on red blood cell deformability *ex vivo*

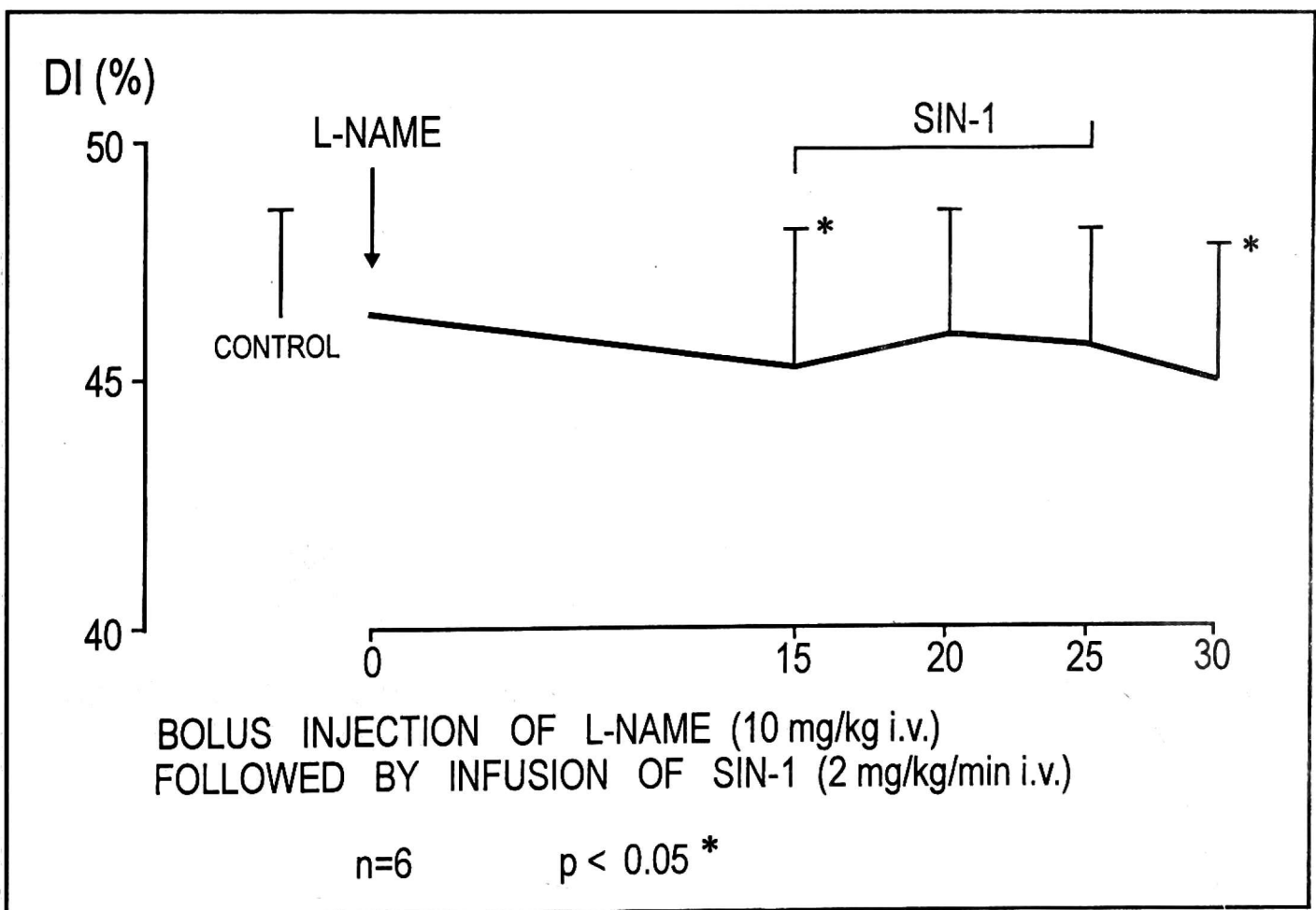


Fig. 4. The effect of SIN-1 on L-NAME-induced red blood cell deformability *ex vivo*

DI (%)

50

45

40

CONTROL

ILOPROST

SIN-1

INCUBATION OF WHOLE BLOOD (open bars) OR ISOLATED ERYTHROCYTES (filled bars) FOR 15 MIN WITH ILOPROST (1 μ M) OR SIN-1 (3 μ M)

n=6

p < 0.05 *
0.01 **

Fig. 5. The effect of iloprost or SIN-1 on red blood cell deformability measured in the suspension of isolated cells or in whole blood *in vitro*

MEA

6

3

0

CONTROL

ILOPROST

SIN-1

INCUBATION OF WHOLE BLOOD (open bars) OR ISOLATED ERYTHROCYTES (filled bars) FOR 15 MIN WITH ILOPROST (1 μ M) OR SIN-1 (3 μ M) IN VITRO

n=6

0.05 *
p < 0.01 **

Fig. 6. The effect of iloprost or SIN-1 on red blood cell aggregability measured in the suspension of isolated cell or in whole blood *in vitro*.

In studies *in vitro* incubation of whole blood or isolated erythrocytes with SIN-1 significantly improved erythrocyte deformability ($48,3\% \pm 2,4$; $n = 6$; $p < 0,01$ vs control $45,9\% \pm 3,4$; $n = 6$ for whole blood and $49,0\% \pm 4,2$; $n = 6$; $p < 0,01$ vs control $45,0\% \pm 3,0$; $n = 6$ for isolated erythrocytes) (Fig. 5) and decreased aggregability ($4,1 \pm 0,9$; $n = 6$; $p < 0,01$ vs control $5,6 \pm 1,1$; $n = 6$ for whole blood and $1,5 \pm 0,6$; $n = 6$; $p < 0,05$ vs control $2,6 \pm 0,9$; $n = 6$ for isolated erythrocytes) (Fig. 6.). Also iloprost improved red blood cell deformability either in whole blood ($48,0\% \pm 3,0$; $n = 6$; $p < 0,01$ vs control $45,9\% \pm 3,4$; $n = 6$) or in isolated red cells ($47,9\% \pm 3,3$; $n = 6$; $p < 0,01$ vs control $45,0\% \pm 3,0$; $n = 6$) (Fig. 5) and diminished aggregation of erythrocytes in whole blood ($4,3 \pm 0,8$; $n = 6$; $p < 0,05$ vs control $5,6 \pm 1,1$; $n = 6$) or in isolated erythrocytes ($1,7 \pm 0,7$; $n = 6$; $p < 0,01$ vs control $2,6 \pm 0,9$; $n = 6$) (Fig. 6).

DISCUSSION

Disturbances in deformability and aggregability of red blood cells were observed in hipercholesterolaemia (7, 15), arterial hypertension (3, 4), diabetes mellitus (5,6) and coronary artery disease (16). In the most of the above studies changes of blood rheology were claimed to result from injury of vascular endothelium with subsequent deficit of endothelium-derived “relaxing” and “antiplatelet” factors, namely nitric oxide (NO) and prostacyclin (PGI_2). In our previous investigations we presented evidences that NO and PGI_2 had modified deformability of red blood cells in septic shock *in vivo* (17) and in physiological conditions *in vitro* (13). Here, for the first time we are showing that these two compounds may affect not only deformability of erythrocytes but also their aggregability. Moreover, exogenous NO is capable of improving blood rheology both in physiological conditions as well as after impairment of rheological properties of blood by L-NAME — the selective inhibitor of NO-synthase which during experiments simply mimics the pathophysiological state of endothelium damage with depletion of endogenous NO. This is why we believe that NO-donors and PGI_2 -analogues, supplementing endogenous generation of NO and PGI_2 , constitute an important pharmacological tool for regulation of blood rheology in health and disease. Indeed, it was shown in clinical studies performed on patients with ischemic heart disease that nitrates induced beneficial changes in red blood cell deformability (18). Beneficial effect of prostacyclin analogue — beraprost — on rheological properties of blood was demonstrated in hypercholesterolaemic rabbits (15).

Along with our observations, clinical studies also revealed unprofitable effect of aspirin on deformability of erythrocytes (19). Interestingly, worsening of deformability by aspirin has been claimed to be not only due to the cyclooxygenase inhibition and prostacyclin depletion but also/or to the

acetylation of integral proteins of the red cell membrane, that led to the rigidizing effect of aspirin on membrane lipid fluidity (20).

What is the mechanism by which NO and PGI₂ regulate deformability and aggregability of red blood cells? Up to now numerous clinical studies (5, 6, 7, 8, 9, 15) as well as our experimental results do not entitle us to answer this question. In our previous work on deformability of rabbit red blood cells *in vitro* (13), erythrocytes and other blood cells could interact freely and that was why we suggested that the deformability of red blood cells might be modulated by the presence of polymorphonuclear leukocytes. However, in *in vitro* experiments reported here, NO and PGI₂ did affect erythrocytes even if they were completely isolated from other blood cells. This allows us to conclude that effects of nitric oxide and prostacyclin on deformability and aggregability of red blood cells are likely not to depend on any other cells. Our speculation that these compounds may affect red cells or/and their membranes directly seems to be quite justified. Undoubtedly further studies are necessary to really settle the problem.

Summarizing, we conclude that L-NAME and aspirin, as measured by their effects on deformability of red blood cells, strongly aggravate rheological properties of blood *in vivo*. Long-lasting damaging effect of L-NAME on erythrocytes can be reversed by exogenous NO. Exogenous NO and prostacyclin are very effective in improving of deformability and in inhibiting of aggregability of red blood cells *in vivo* and *in vitro*, both in whole blood as well as in suspension of isolated cells. NO and prostacyclin seem to affect red blood cells directly and the presence of other blood cells is not required for such action. NO-donors and prostacyclin analogues may prove useful in the treatment of microcirculatory disturbances or hyperviscosity syndromes.

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