Human platelets perfusion through isolated guinea-pig heart: the effects on coronary flow and oxidative stress markers

Slobodan Novokmet[1], Vladimir Lj. Jakovljevic[2], Slobodan Jankovic[3], Goran Davidovic[4], Nebojsa Andjelkovic[4], Zvezdan Milanovic[5] and Dragan M. Djuric[6]

[1] *Department of Pharmacy, Faculty of Medicine, University of Kragujevac, Serbia*
[2] *Department of Physiology, Faculty of Medicine, University of Kragujevac, Serbia*
[3] *Department of Pharmacology, Faculty of Medicine, University of Kragujevac, Serbia*
[4] *Internal Clinic, CC Kragujevac, Faculty of Medicine, University of Kragujevac, Serbia*
[5] *Department of Physiology, Faculty of Medicine, Kosovska Mitrovica, Serbia*
[6] *Institute of Medical Physiology, School of Medicine, University of Belgrade, Serbia*

Abstract. Present study was designed to evaluate effect of perfusion with human platelets reach plasma (PRP) on coronary flow (CF) and oxidative stress markers in coronary vascular bed of the isolated guinea-pig heart. In coronary venous effluent the following oxidative stress markers were estimated: nitrite as a measure of nitric oxide (NO) production, superoxide anion (O_2^-), and index of lipid peroxidation (TBARS). Isolated guinea-pig hearts (n = 6, b.m. 250–300 g) were perfused according to a Langendorff's technique at different (30, 70, and 120 cmH_2O) coronary perfusion pressures (CPP). Samples were collected at control conditions and during perfusion with platelets rich plasma (PRP) obtained either from healthy volunteers or from patients with acute myocardial infarction (PRP (AMI)) with/or without previous inhibition of NO synthase (NOS) by Nω-nitro-L-arginine monomethyl ester (L-NAME, 30 μmol/l). PRP and PRP (AMI) perfusion induced reduction of CF and all evaluated oxidative stress parameters. The reduction of CF was more potentiated in PRP (AMI) as in PRP group, while oxidative stress parameters where significantly decreased only in PRP (AMI). In addition, previous blockade of NOS by L-NAME potentiated these effects only in PRP (AMI) group. It can be concluded that non-activated and activated platelets interact with coronary endothelium in similar way, with more significant influence of activated platelets on CF and oxidative stress markers.

Key words: Platelets reach plasma — Guinea-pig heart — Coronary flow — Oxidative stress

Introduction

Platelet-endothelium interaction represents a common mechanism in normal circulating blood, as well as mechanism of homeostasis regulation (Furie et al. 2008). Activation of platelets is tightly regulated, thus, oxidative stress can alter platelets responses and inhibit their function (Freedman 2008). The importance of platelet-vessel wall interactions in ischemic heart disease has been proved by certain studies demonstrating a protective effect of some antiplatelet drugs in patients with unstable angina and with myocardial infarction (Tantry et al. 2005; Scharf et al. 2008). Although there may be several distinct causes of myocardial ischemia, there is good evidence for platelet involvement in some of its main clinical manifestations (Takaya et al. 2005; Scharf 2008). Activated platelets in acute myocardial infarction provide specific reactions, including their role in coronary vasospasm and have very important effects on endothelium (Konidala et al. 2004). It is known that platelets contribute to ischemia- and reperfusion-induced injury of the heart (Seligmann et al. 2000), and in the coronary system can impair cardiac pump function and postischemic recovery of external heart work (Heindl et al. 1999). Nitric oxide (NO) provides protection effects on platelets and plays an important role as inhibitor of platelet activation in the coronary circulation (Pohl and

Correspondence to: Dragan M. Djuric, Institute of Medical Physiology, School of Medicine, University of Belgrade, Višegradska 26/II, 11000 Belgrade, Serbia
E-mail: drdjuric@eunet.rs

Busse 1989), without significant influence on platelet count in blood (Lowson et al. 1999).

The present study was designed to evaluate the effects of perfusion of human platelet rich plasma from healthy volunteers (PRP group) vs. PRP from patients with acute myocardial infarction (PRP (AMI) group) on coronary flow (CF) and oxidative stress markers during nitric oxide synthase (NOS) inhibition through coronary vascular bed of the isolated guinea-pig heart.

Materials and Methods

Isolated guinea-pig heart preparation

Guinea-pigs of either sex (body weight 250–300 g) were killed by cervical dislocation according to Schedule 1 of the Animals, Scientific procedures (Act 1986, UK), without use of any anticoagulants or anesthetics. After urgent thoracotomy and rapid arrest of the beating hearts by superfusion with ice-cold isotonic saline, the hearts were isolated and perfused according to the Langendorff's technique. The composition of the Krebs-Henseleit buffer (perfusion medium) was as follows (in mmol/l): NaCl 118; KCl 4.7; $CaCl_2 \times 2H_2O$ 2.5; $MgSO_4 \times 7H_2O$ 1.7; $NaHCO_3$ 25; KH_2PO_4 1.2; glucose 11; pyruvate 2. It was equilibrated with gas mixture (5% CO_2 – 95% O_2) at 37°C, (pH 7.4). All hearts were electrically paced (5 V) at constant rate of 320 bpm (beats/minute). Constant left ventricular draining through the dissected mitral valve was performed.

Physiological assay

Stabilization of preparation was performed at basal coronary perfusion pressure (CPP) of 70 cmH_2O for 30 min. During this period, the hearts were challenged by short-term occlusions (5–15 s). At the end of equilibration period, CPP was lowered to 30 cmH_2O (to mimic ischemia), then increased gradually in the reverse order to 70 cmH_2O (normal conditions), and further to 120 cmH_2O (to mimic shear stress). Myocardial perfusion was established at constant flow for all applied CPP. When flow was considered as stable at each value of perfusion pressure, samples of the coronary venous effluent were collected. At the end of this series of pressure changes (basic protocol), hearts were perfused with PRP, prepared from venous blood of healthy volunteers. In the second series of experiments, the basic protocol was followed by perfusion with PRP, prepared from venous blood of patients with acute myocardial infarction-early phase (PRP (AMI)). In the third series of experiments, hearts were perfused with Nω-nitro-L-arginine monomethyl ester (L-NAME, 30 μmol/l – final concentration), as an inhibitor of NOS (Emery 1995) from the beginning of the experiment and, after the first sequence of perfusion pressure changes protocol was repeated in the presence of PRP+L-NAME (30 μmol/l). The fourth series of experiments were the same as the third but PRP (AMI) was administered instead of PRP.

Preparation of PRP

Venous blood was obtained from healthy volunteers who had not received any medications as well as from the patients with acute myocardial infarction in early phase who had no received any anti-aggregating drugs; then it was collected into 3.8% sodium citrate (20% of final volume). PRP was obtained according to described method (Schrör et al. 1981) by centrifugation at 1000 g for 20 min, with previous incubation at room temperature for 15 min. The infusion of PRP and PRP (AMI) were performed, at constant rate of 1 ml/min for 5 min (each heart), as well as after administration of L-NAME (final concentration of 30 μmol/l; Merck, Darmstadt, Germany).

Biochemical assays

Samples of coronary venous effluent were collected after the stabilization of flow at each perfusion pressure value.

Nitrite determination

NO was assessed as nitrite and quantified by the spectrophometric method using the Griess-reagent. 0.5 ml of perfusate was precipitated with 200 μl of 30% sulfosalicylic acid, vortexed for 30 min and centrifuged at 3000 × *g*. Equal volumes of the supernatant and Griess reagent, containing 1% sulfanilamide in 5% phosphoric acid / 0.1% napthalene ethylenediamine-dihydrochloride was added and incubated for 10 min in the dark and read at 543 nmol/l. The nitrite levels were calculated by using sodium nitrite as a standard (Green et al. 1982).

Superoxide determination

The level of superoxide anion (O_2^-) was measured using NBT (nitro blue tetrazolium) reaction in Tris-buffer with coronary venous effluent and read at 530 nm. Krebs-Hensenleit solution was used as a blank probe (Auclair and Voisin 1985).

Index of lipid peroxidation (TBARS)

The degree of lipid peroxidation in coronary venous effluent was estimated by measuring of thiobarbituric acid reactive substances (TBARS) using 1% TBA in 0.05 NaOH incubated with coronary effluent at 100°C for 15 min and read at 530 nm. Krebs-Hensenleit solution was used as a blank probe (Ohkawa et al. 1979).

Statistical analysis

Values are expressed as means ± SEM. Statistical analysis was performed by using multifactorial analysis of variance for repeated measurements between subject factors as well as Bonferroni test. p values less than 0.05 were considered to be significant.

Results

PRP perfusion

PRP perfusion induced significant reduction of CF vs. control at all applied CPP (30, 70 and 120 cmH_2O): from 32% at 30 cmH_2O to 27% at 120 cmH_2O. On the other hand, only nitrite was significantly reduced under the influence of PRP: 46% at 70 cmH_2O and 28% at 120 cmH_2O. The level of oxidative stress markers was not significantly influenced by PRP (Figure 1A and Table 1).

PRP (AMI) perfusion

PRP (AMI) perfusion induced reduction of CF and oxidative stress markers vs. control at all applied CPP (30, 70 and 120 cmH_2O). The reduction increased at higher CPP values for CF and for oxidative stress markers: from 39% at 70 cmH_2O to 44% at 120 cmH_2O for CF, 42% at 70 cmH_2O to 35% at 120 cmH_2O for nitrite, 34% at 70 cmH_2O to 43% at 120 cmH_2O for O_2^-, and 90% at 70 cmH_2O to 74% at 120 cmH_2O for TBARS (Figure 1B and Table 2).

PRP perfusion plus L-NAME

PRP perfusion plus L-NAME (30 μmol/l), did not induced additional reduction of CF and oxidative stress markers

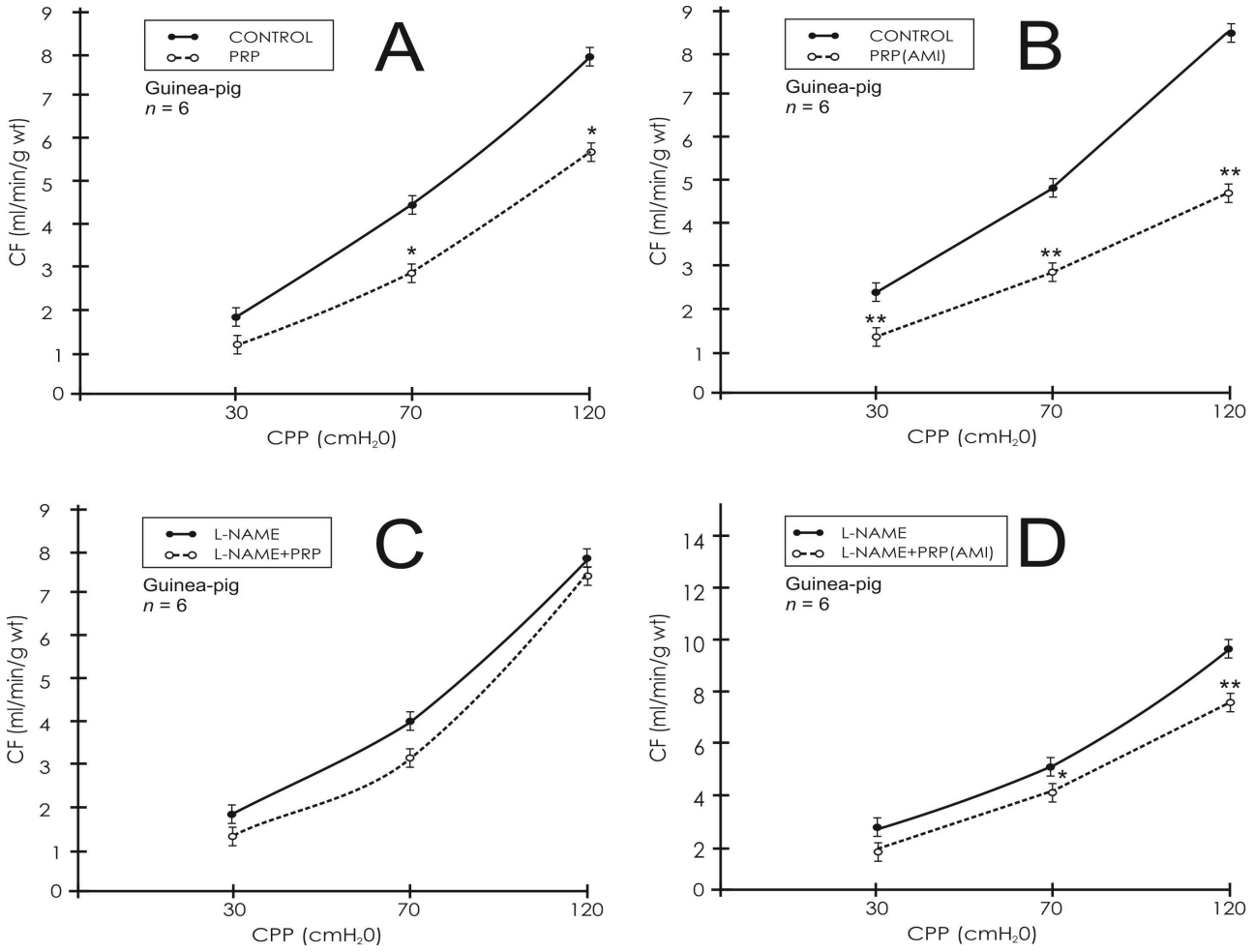

Figure 1. The effects of PRP or PRP (AMI) passage through isolated guinea-pig heart on CF alone (A, B) or in the presence of L-NAME (30 μmol/l) (C, D). *$p < 0.05$, **$p < 0.01$

Table 1. The effects of PRP perfusion through isolated guinea-pig heart on CF, nitrite, O_2^- and TBARS outflow

$n = 6$	CPP (cmH_2O)	CF (ml/min × g wt ± SE)	Nitrite (nmol/min × g wt ± SE)	O_2^- (nmol/min × g wt ± SE)	TBARS (nmol/min × g wt ± SE)
Control	30	1.960 ± 0.210	0.137 ± 0.041	5.296 ± 1.003	166.358 ± 16.117
	70	4.520 ± 0.351	0.664 ± 0.072	18.098 ± 5.023	323.552 ± 53.057
	120	7.960 ± 0.714	1.273 ± 0.061	27.854 ± 5.259	510.070 ± 138.971
PRP	30	1.340 ± 0.210*	0.102 ± 0.041	2.584 ± 1.003	70.316 ± 16.117
	70	3.000 ± 0.351*	0.355 ± 0.072**	13.248 ± 5.023	215.376 ± 53.057
	120	5.800 ± 0.714*	0.918 ± 0.061**	24.182 ± 5.259	319.982 ± 138.971

* $p < 0.05$, ** $p < 0.01$ – PRP vs. control.

Table 2. The effects of PRP (AMI) perfusion through isolated guinea-pig heart on CF, nitrite, O_2^- and TBARS outflow

$n = 6$	CPP (cmH_2O)	CF (ml/min × g wt ± SE)	Nitrite (mol/min × g wt ± SE)	O_2^- (nmol/min × g wt ± SE)	TBARS (nmol/min × g wt ± SE)
Control	30	2.475 ± 0.394	0.146 ± 0.030	2.613 ± 0.216	121.835 ± 16.715
	70	4.900 ± 0.509	0.350 ± 0.084	5.913 ± 0.530	371.580 ± 75.849
	120	8.500 ± 0.760	0.435 ± 0.070	8.795 ± 0.462	430.270 ± 42.575
PRP (AMI)	30	1.450 ± 0.394**	0.112 ± 0.030	1.500 ± 0.249	27.503 ± 16.715
	70	2.975 ± 0.509**	0.204 ± 0.084**	3.903 ± 0.612**	37.053 ± 75.849**
	120	4.800 ± 0.760**	0.284 ± 0.070**	5.057 ± 0.533**	109.257 ± 42.575**

* $p < 0.05$, ** $p < 0.01$ – PRP (AMI) vs. control.

Table 3. The effects of PRP perfusion through isolated guinea-pig heart with previous inhibiton of endothelial NOS (L-NAME 30 μmol/l) on CF, nitrite, O_2^- and TBARS outflow

$n = 6$	CPP (cmH_2O)	CF (ml/min × g wt ± SE)	Nitrite (nmol/min × g wt ± SE)	O_2^- (nmol/min × g wt ± SE)	TBARS (nmol/min × g wt ± SE)
L-NAME	30	1.860 ± 0.210	0.255 ± 0.032	4.490 ± 1.396	161.698 ± 39.300
	70	3.540 ± 0.314	0.494 ± 0.054	13.526 ± 3.930	326.762 ± 56.603
	120	6.580 ± 0.358	1.133 ± 0.084	21.822 ± 5.253	656.224 ± 130.485
L-NAME+PRP	30	1.475 ± 0.235	0.230 ± 0.036	3.185 ± 1.561	122.215 ± 43.939
	70	2.800 ± 0.351	0.453 ± 0.060	9.720 ± 4.394	220.498 ± 63.284
	120	6.250 ± 0.400	1.025 ± 0.094	21.908 ± 5.873	547.677 ± 145.887

Table 4. The effects of PRP (AMI) perfusion through isolated guinea-pig heart with previous inhibiton of endothelial NOS (L-NAME 30 μmol/l) on CF, nitrite, O_2^- and TBARS outflow

$n = 6$	CPP (cmH_2O)	CF (ml/min × g wt ± SE)	Nitrite (nmol/min × g wt ± SE)	O_2^- (nmol/min × g wt ± SE)	TBARS (nmol/min × g wt ± SE)
L-NAME	30	2.650 ± 0.195	0.07625 ± 0.005	4.852 ± 0.632	279.870 ± 20.223
	70	5.125 ± 0.272	0.179 ± 0.003	11.470 ± 1.459	413.298 ± 21.355
	120	9.675 ± 0.269	0.374 ± 0.006	21.235 ± 0.893	624.720 ± 30.779
L-NAME+PRP (AMI)	30	1.950 ± 0.195*	0.0492 ± 0.005	2.978 ± 0.632	217.035 ± 20.223
	70	4.225 ± 0.272*	0.147 ± 0.003*	9.393 ± 1.459	325.270 ± 21.355*
	120	7.550 ± 0.269**	0.292 ± 0.006*	16.548 ± 0.893*	487.415 ± 30.779*

* $p < 0.05$, ** $p < 0.01$ – L-NAME+PRP (AMI) vs. L-NAME.

compared to L-NAME alone at all applied CPP (Figure 1C and Table 3).

PRP (AMI) perfusion plus L-NAME

PRP (AMI) perfusion plus L-NAME (30 μmol/l) induced additional reduction of CF and oxidative stress markers vs. L-NAME alone at all applied CPP (30, 70 and 120 cmH_2O). The reduction was more pronounced at lower CPP values for CF and oxidative stress markers except for O_2^-: from 42% at 30 cmH_2O to 34% at 120 cmH_2O for CF, 50% at 30 cmH_2O to 34% at 120 cmH_2O for nitrite, 51% at 30 cmH_2O to 66% at 120 cmH_2O for O_2^-, and 40% at 30 cmH_2O to 34% at 120 cmH_2O for TBARS (Figure 1D and Table 4).

Discussion

Platelets-induced myocardial dysfunction in ischemic and reperfused guinea-pig hearts is mediated by reactive oxygen species (ROS) (Seligmann et al. 2002). Univalent reduction of molecular oxygen and univalent oxidation of hydrogen peroxide, in biological systems, provides formation of O_2^- (Fridovich 1998). The toxicity of O_2^- has been based on direct interaction with cells or its components which is the most common biochemical effect of O_2^- (Brüne et al. 1991). Lipid peroxidation is a degenerative process which is indicated by formation of TBARS (Dotan et al. 2004). Lipid peroxidation includes oxidative damage in cell membranes, lipoproteins, and other lipid-containing structures, and it has been linked to a variety of disorders, including atherogenesis and ischemia-reperfusion injury (Girotti 1998).

It is possible that response of endothelium to oxidative stress is dependent on platelet oxidant or antioxidant status (Freedman 2008). In addition to directly activating platelets or decreasing the threshold for platelet activation, O_2^- reacts with platelets or endothelium-derived NO forming $ONOO^-$, which is of particular importance for endothelial dysfunction and thrombosis (Krötz et al. 2004). This is primarily caused by the decreased bioavailability of NO as a potent inhibitor of platelet activation (de Belder et al. 1994). However, NO under physiological conditions prevents platelet activation by increasing cGMP levels (Moncada et al. 1991). It is not surprising, therefore, that the antithrombotic effect of NO is lost either when ROS are exogenously added to the system or when they are scavenged (Moncada et al. 1991).

Overall, there is accumulating evidence supporting a net prothrombotic effect of vascular-derived and platelet-derived ROS *in vitro* (Krötz et al. 2004). Taken together, these data point to an important role of platelet-derived ROS and the intraplatelet redox state in the regulation of physiological platelet activation (Krötz et al. 2004). In particular, the specific significance of the different types of ROS with respect to platelet activity has not definitely been determined (Ovechkin et al. 2007). Although it is clearly evident that ROS participate in platelet activation and subsequent thrombus formation, it is not fully understood whether ROS serve as indispensable signaling molecules for specific platelet activation pathways (Krötz et al. 2004).

Our results clearly show that during control conditions increased CF might be observed following increased CPP. It was probably a consequence of higher shear stress and increased NO production (Yan et al. 2007). PRP decreased CF at all applied perfusion pressures compared to the control, significantly (Figure 1A). Furthermore, perfusion with PRP (AMI) decreased CF at all CPP values more powerfull compared to PRP group (Figure 1B). The fact that CF drop was more pronounced in PRP (AMI) group was probably due to the endothelial stimulation by platelets (and/or plasma) enriched with more vasoconstrictor substances producing significant vasoconstriction (Cines et al. 1998). Also a relative insufficiency of coronary vasodilator mechanisms in contact with PRP (AMI) may not be exluded (Worthley et al. 2007). Thus, different substances like tromboxanes, prostaglandines, serotonin, ROS etc. which interact with endothelium might be released from platelets (Kleiman et al. 2008). In addition if NOS inhibitor (i.e. L-NAME) was administered to the medium, the curve of CF was shifted to the right indicating decreased CF and probably decreased NO synthesis/production (Jakovljevic et al. 2003). However, it has been proposed that perfusion of PRP or PRP (AMI) might elicit pressure-dependent NO release into coronary effluent which was less pronounced in PRP (AMI) group (Sun et al. 2004). Therefore, L-NAME blocked NO release in both experimental groups. When L-NAME was applied in concentration high enough to block NO synthesis, there was still some NO release persisting (Emery 1995). There are two posibilities: i) the concentration of L-NAME applied was not so sufficient to completely block NO synthesis; ii) NO could be probably produced from other cells than endothelium, i.e. vascular smooth muscle cells, platelets etc. (Vallance et al. 2002). In addition, when PRP (AMI) was administered in presence of L-NAME further decrease in CF suggests that the mechanisms other than decreased NO production may have been included in vasoconstriction (Just et al. 2005). Therefore, specific antagonists should be used in further experiments to evaluate these vasoconstrictor mechanisms and potential role in coronary vasospasm.

It can be concluded that PRP (AMI) which was obtained from the patients with acute myocardial infarction have more vasoconstrictor potential than platelets of healthy volunteers, similar like in recent studies (Brodov et al. 2008). There is significant PRP (AMI) ability to decrease CF and NO release. In our study blocking of NO synthesis

with L-NAME confirm this conclusion in paralell. However, there are some limitations of applied methodology. Activated platelets in acute myocardial infarction provided basic reactions and have important role for endothelium (Heindl et al. 1998a,b, 1999).

PRP administration induced reduction of CF, nitrite, superoxide, and TBARS outflow at all applied CPP values. This reduction was significant only for CF and nitrite, but not for oxidative stress markers. These data let us to conclude that estimated oxidative stress markers don't have role in platelet-endothelium interaction, considering platelets obtained from blood of healthy volunteers and not damaged vascular endothelium.

The administration of PRP (AMI) resulted in reduction of oxidative stress markers at 70 and 120 cmH_2O. Previous administration of L-NAME potentiated these reductions for CF and nitrite, considering PRP (AMI) administration, what was not a case with PRP administration. Although studies with washed platelets allow for "unaltered" information about the function of a specific ROS, this scenario is not necessarily representative of physiological conditions, because plasma or full blood possesses antioxidant capacities (Krötz et al. 2004). Thus, awareness of the assay conditions is of importance for the interpretation of data regarding the influence of ROS on platelets-endothelium interaction (Cominacini et al. 2003). If the influence of a specific ROS on platelets is investigated *in vitro*, then buffers that do not contain antioxidant substances may be appropriate (Krötz et al. 2004). If consequences of such signaling should be assessed with relevance for the *in vivo* situation, however, then the effects of addition of defined amounts of plasma or full blood should be tested (Krötz et al. 2004).

In summary, more significant decrease of oxidative stress markers in the presence of PRP (IAM) as in PRP group schould be the effect of increased plasma antioxidative capacities, induced by activated platelets. Additional influence of NOS blockade only in PRP (IAM), but not in PRP treated hearts suggested potential role of L-arginine/NO system on oxidative stress by previous activitation of circulating platelets.

Acknowledgement. This work was supported by grant No. 145014G of the Ministry of Science and Environmental Protection of Republic of Serbia.

References

Auclair C., Voisin E. (1985): Nitroblue tetrazolium reduction. In: Handbook of Methods for Oxygen Radical Research. (Ed. R. A. Greenwald), pp. 123–132, CRC Press, Boca Raton, Florida

Brodov Y., Sandach A., Boyko V., Matetzky S., Guetta V., Mandelzweig L., Behar S. (2008): Acute myocardial infarction preceded by potential triggering activities: angiographic and clinical characteristics. Int. J. Cardiol. **130**, 180–184

Brüne B., von Appen F., Ullrich V. (1991): Oxidative stress in platelets, in oxidants and antoxidants. In: Oxidative Stress. Oxidants and Antioxidants. (Ed. H. Siess), pp. 422–443, Academic Press, San Diego

Cominacini L., Pasini A. F., Garbin U., Pastorino A., Rigoni A., Nava C., Davoli A., Lo Cascio V., Sawamura T. (2003): The platelet-endothelium interaction mediated by lectin-like oxidized low-density lipoprotein receptor-1 reduces the intracellular concentration of nitric oxide in endothelial cells. J. Am. Coll. Cardiol. **41**, 499–507

Cines D. B., Pollak E. S., Buck C. A., Loscalzo J., Zimmerman G. A., McEver R. P, Pober J. S., Wick T. M., Konkle B. A., Schwartz B. S., Barnathan E. S., McCrae K. R., Hug B. A., Schmidt A.-M., Stern D. M. (1998): Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood **91**, 3527–3561

de Belder A. J., MacAllister R., Radomski M. W., Moncada S., Vallance P. J. T. (1994): Effects of S-nitroso-glutathione in the human forearm circulation: evidence for selective inhibition of platelet activation. Cardiovasc. Res. **28**, 691–694

Dotan Y., Lichtenberg D., Pinchuk I. (2004): Lipid peroxidation cannot be used as a universal criterion of oxidative stress. Prog. Lipid Res. **43**, 200–227

Emery C. J. (1995): Vasodilatator action of the S-nitrosothiol, SNAP, in rat isolated perfused lung. Physiol. Res. **44**, 19–24

Freedman J. E. (2008): Oxidative stress and platelets. Arterioscler. Thromb. Vasc. Biol. **28**, S11–16

Fridovich I. (1998): Oxygen toxicity: a radical explanation. J. Exp. Biol. **201**, 1203–1209

Furie B., Furie B. C. (2008): Mechanisms of thrombus formation. N. Engl. J. Med. **359**, 938–949

Girotti A. W. (1998): Lipid hydroperoxide generation, turnover, and effector action in biological systems. J. Lipid. Res. **39**, 1529–1542

Green L. C., Wagner D. A., Glogowski J., Skipper P. L., Wishnok J. S., Tannenbaum S. R. (1982): Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. Anal. Biochem. **126**, 131–138

Heindl B., Becker B. F., Zahler S., Conzen P. F. (1998a): Volatile anaesthetics reduce adhesion of blood platelets under low-flow conditions in the coronary system of isolated guinea pig hearts. Acta Anaesthesiol. Scand. **42**, 995–1003

Heindl B., Zahler S., Welsch U., Becker B. F. (1998b): Disparate effects of adhesion and degranulation of platelets on myocardial and coronary function in postischaemic hearts. Cardiovasc. Res. **38**, 383–394

Heindl B., Conzen P. F., Becker B. F. (1999): The volatile anesthetic sevoflurane mitigates cardiodepressive effects of platelets in reperfused hearts. Basic Res. Cardiol. **94**, 102–111

Jakovljevic V. Lj., Ignjatovic V. S., Djurdjevic P. M., Tasic M. V., Jovovic D. M., Djuric D. M. (2003): The effects of nimodipine on coronary flow, nitrite outflow and superoxide anion release in isolated rat heart. Am. J. Hypertens. **16**, A74

Just A., Olson A. J. M., Falck J. R., Arendshorst J. R. (2005): NO and NO-independent mechanisms mediate ETB receptor buffering of ET-1-induced renal vasoconstriction in the rat. Am. J. Physiol., Regul. Integr. Comp. Physiol. **288**, R1168–1177

Kleiman N. S., Freedman J. E., Tracy P. B., Furie B. C., Bray P. F., Rao S. V., Phillips D. R., Storey R. F., Rusconi C. P., French P. A., Steinhubl S. R., Becker R. C. (2008): Platelets: developmental biology, physiology, and translatable platforms for preclinical investigation and drug development. Platelets **19**, 239–251

Konidala S., Gutterman D. D. (2004): Coronary vasospasm and the regulation of coronary blood flow. Prog. Cardiovasc. Dis. **46**, 349–373

Krötz F., Sohn H.-Y., Pohl U. (2004): Reactive oxygen species: players in the platelet game. Arterioscler. Thromb. Vasc. Biol. **24**, 1988–1996

Lowson S. M., Hassan H. M., Rich G. F. (1999): The effect of nitric oxide on platelets when delivered to the cardiopulmonary bypass circuit. Anesth. Analg. **89**, 1360–1365

Moncada S., Palmer R. M., Higgs E. A. (1991): Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. **43**, 109–142

Ohkawa H., Ohishi N., Yagi K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. **95**, 351–358

Ovechkin A. V., Lominadze D., Sedoris K. C., Robinson T. W., Tyagi S. C., Roberts A. M. (2007): Lung ischemia-reperfusion injury: implications of oxidative stress and platelet-arteriolar wall interactions. Arch. Physiol. Biochem. **113**, 1–12

Pohl U., Busse R. (1989): EDRF increases cyclic GMP in platelets during passage through the coronary vascular bed. Circ. Res. **65**, 1798–1803

Scharf R. E. (2008): Acquired platelet function disorders – pathogenesis, classification, frequency, diagnosis, clinical management. Hämostaseologie **28**, 299–311 (in German)

Schrör K., Köhler P., Müller M. (1981): Prostacyclin-tromboxane interactions in the platelet-perfused *in vitro* heart. Am. J. Physiol., Heart Circ. Physiol. **241**, H18–25

Seligmann C., Schimmer M., Lietsch T., Bock A., Simsek C. (2000): A thrombocyte-induced myocardial dysfunction in the ischemic and reperfused guinea pig heart is mediated by reactive oxygen species. Free. Radic. Biol. Med. **29**, 1244–1251

Seligmann C., Simsek Y., Schimmer M., Leitsch T., Bock A., Schultheiss H. P. (2002): Human thrombocytes are able to induce a myocardial dysfunction in the ischemic and reperfused guinea pig heart mediated by free radicals – role of the GPIIb/IIIa-blocker tirofiban. Life Sci. **71**, 2319–2329

Sun D., Huang A., Kaley G. (2004): Mechanical compression elicits NO-dependent increases in coronary flow. Am. J. Physiol., Heart Circ. Physiol. **287**, H2454–2460

Takaya N., Katoh Y., Iwabuchi K., Hayashi I., Konishi H., Itoh S., Okumura K., Ra C., Nagaoka I., Daida H. (2005): Platelets activated by collagen through the immunoreceptor tyrosine-based activation motif in the Fc receptor γ-chain play a pivotal role in the development of myocardial ischemia-reperfusion injury. J. Mol. Cell. Cardiol. **39**, 856–864

Tantry U. S., Bliden K. P., Gurbel P. A. (2005): Resistance to antiplatelet drugs: current status and future research. Expert Opin. Pharmacother. **6**, 2027–2045

Vallance P., Leiper J. (2002): Blocking NO synthesis: how, where and why? Nat. Rev. Drug Discov. **1**, 939–950

Worthley M. I., Holmes A. S., Willoughby S. R., Kucia A. M., Heresztyn T., Stewart S., Chirkov Y. Y., Zeitz C. J., Horowitz J. D. (2007): The deleterious effects of hyperglycemia on platelet function in diabetic patients with acute coronary syndromes. Mediation by superoxide production, resolution with Intensive insulin administration. J. Am. Coll. Cardiol. **49**, 304–310

Yan C., Huang A., Kaley G., Sun D. (2007): Chronic high blood flow potentiates shear stress-induced release of NO in arteries of aged rats. Am. J. Physiol., Heart Circ. Physiol. **293**, H3105–3110