K. BIEROŃ, J. ŚWIĘS, E. KOSTKA-TRĄBKA, R. J. GRYGLEWSKI

THROMBOLYTIC AND ANTIPLATELET ACTION OF XANTHINOL NICOTINATE (SADAMIN): POSSIBLE MECHANISMS

Chair of Pharmacology, Jagiellonian University Medical School, Cracow, Poland.

Here we report on thrombolytic and hypotensive actions of Xanthinol nicotinate (Sadamin) in rats and on its anti-platelet and fibrinolytic effects in patients with peripheral arterial obliterative disease (PAOD). Special consideration was given to a proposal of new mechanisms of anti-platelet and thrombolytic actions of Sadamin. We conclude that the mechanism of anti-platelet and thrombolytic activity of Sadamin partly consists of a simultaneous release of endogenous prostacyclin and nitric oxide by the nicotinate component of Sadamin, whereas the theophylline component is responsible for enhancement of physiological actions of these endothelial mediators at the level of cyclic nucleotides which are their second messengers.

K e y w o r d s: *xantinol nicotinate, prostacyclin, nitric oxide, thrombolysis, hypontensive action, antiplatelet activity, cAMP, cGMP*

INTRODUCTION

Xanthinol nicotinate 7-{2-hydroxy-3-[(2-hydroxyethyl)-methylamine]-propyl}-theophylline nicotinate, (Sadamin "Polfa" Cracow) comprises two components represented by moities of teophylline derivative and nicotinic acid. Both components are responsible for a known vasorelaxant action of Sadamin; nicotinate moiety seems to act through endogenous endothelial mediators (1—3) while theophylline is supposed to reinforce actions of the released endothelial mediators *via* inhibition of intracellular phosphodiesterases in vascular myocytes, in this way, increasing levels of cyclic nucleotides (4, 5). Therefore, Sadamin improves tissue perfusion. Apart from its vascular action Sadamin is known to lower cholesterol blood level (6). These properties of Sadamin are usually quoted to explain its effectiveness in the treatment of patients with peripheral arterial obliterative disease (PAOD) (7).

Here we report on a new way of action of Sadamin in PAOD patients, i.e. on its anti-platelet and fibrinolytic properties, the mechanism of which we have tried to rationalize in animal experiments.

MATERIALS AND METHODS

THROMBOLYSIS IN VIVO

Thrombolytic and hypotensive actions of Sadamin in rats

The evaluation of thrombolytic activity of Sadamin was performed *in vivo* in anaesthetized rats with extracorporeal circulation (8). Briefly animals (male Wistar rats) weighing from 250 to 400g were anaesthetized with thiopental (100 mg/kg i.p.) and heparinized (800 U/kg i.v.). For the monitoring of the mean arterial pressure the Isotec type electronic transducer was connected with a cannula to the left carotid artery, whereas the cannulated right carotid artery was delivering blood into the extracorporeal circulation by means of a peristaltic pump (37 °C, 1 ml/min). The withdrawn blood superfused the isolated collagen strip. Following superfusion of the collagen strip the blood returned into the circulation through the cannulated right femoral vein.The weight of the superfused strip is continuously monitored by means of the modified 386 Harvard transducer. During superfusion a clot was formed on the surface of the collagen strip. As confirmed by the electron microscopy the clot consists mainly of platelet aggregates, a few erytrocytes, leucocytes and scanty patches of fibrin. The formation of the clot resulted in gain of weight that was proportional to the size of the clot, and after 20—30 min reached plateau. Intravenous injections of prostacyclin or its analogue iloprost produced thrombolysis as shown a loss in weight of the preformed thrombi which were attached to the blood-superfused strips.

The following drugs were given intravenously: Sadamin („Polfa" Cracow) 30 mg/kg, a COX-1 inhibitor, indomethacin (Sigma) 10 mg/kg, an inhibitor of nitric oxide synthase — N^ω-nitro-L-arginine methyl ester (L-NAME, Sigma). Indomethacin or L-NAME were administered 10 min before injection of Sadamin.

ANTIPLATELET AND FIBRINOLYTIC ACTION OF SADAMIN IN PAOD PATIENTS EX VIVO

20 male PAOD patients aged 41—69 years (mean 55 years) entered this study. Sadamin was dissolved in 200 ml of saline and given intravenously at a dose 10 mg/kg during 3 h lasting infusions twice a day with 12 h intervals for 21 days. Before and after infusions of Sadamin on 1 st, 10 th and 21 st day the following tests were performed:

1. Assay of threshold proaggregatory concentrations for adenosine diphosphate (ADP) according to the Born's method (9)
2. Assay of spontaneous platelet aggregability expressed as platelet aggregates ratio (PAR) using the Wu and Hoak method (10)
3. Euglobulin clot lysis time (ECLT) according to von Kaulla (11).

Platelet aggregability in PAOD patients

ADP-induced platelet aggregation

Venous blood which contained tri-sodium citrate (3.8% v/v) at a ratio 9:1 was centrifuged at room temperature at $200 \times g$ or at $2000 \times g$ for 10 min. In this way platelet rich plasma (PRP) or platelet poor plasma (PPP) was obtained respectively. The platelet count in PRP was adjusted with homologous PPP to a count of 2×10^8 platelets/ml. The threshold pro-aggregatory concentrations of ADP were determined in a Born aggregometer and were expressed as EC_{30} of ADP-induced aggregation.

Spontaneous platelet aggregability

Blood samples (0.5 ml) were drawn from the antecubital vein directly into two separate polypropylene syringes. One contained 2 ml of buffered ethylenediaminetetraacetic acid (EDTA) formalin solution and the other 2 ml of isotonic buffered EDTA solution at pH 7.4. After thorough mixing the contents were transfered to two polypropylene tubes and centrifuged at $200 \times g$ for 10 min at 22 °C to obtain PRP. Platelet counts in both samples were determined in a Bürker chamber and the results were expressed as the ratio of platelet aggregates (PAR) i.e. the ratio of the platelet count in a mixture of EDTA-formalin-PRP to the platelet count in a mixture of EDTA-PRP.

Fibrinolytic action in PAOD patients — euglobulin clot lysis time (ECLT)

Venous blood which contained tri-sodium citrate (3.8% v/v) at a ratio 4:1 was centrifuged at room temperature at $2000 \times g$ for 5 min to produce PPP. Distilled water was added (14 ml/ml of PPP) and the pH was adjusted to 5.4 by bubbling with CO_2 gas. This procedure causes precipitation of the euglobulin fraction, while the acidity destroys the biological activity of plasminogen activator inhibitor (PAI-1). After a second centrifugation at $2000 \times g$ for 5 min the supernatant was discarded and the euglobulin precipitate was dissolved in 1 ml of phosphate buffer (13.4 mM KH_2PO_4/53.6 mM Na_2HPO_4). To induce clotting 10 μl of solution of 200 U thrombin/ml in 0.05 M $CaCl_2$ was added. The clot was incubated at 37 °C and the time required for complete lysis was recorded.

Statistical analysis

All data are expressed as the means $\pm$ SEM of n experiments. Statistical analysis was performed using paired Student's t-test and p values were calculated ($p < 0.05$ were considered as significant).

RESULTS

Thrombolytic and hypotensive actions of Sadamin in rats

In rats Sadamin at a dose of 30 mg/kg i.v. produced loss in weight of thrombus by an immediate (after 3—4 min) and short-lasting (< 1 h) thrombolytic effect (29—31%) (*Table 1*) and lowering of mean arterial blood pressure by 22—25%. (*Table 2*).

Thrombolytic (but not hypotensive) effect of Sadamin was significantly reduced by pretreatment with a cyclooxygenase-1 inhibitor, indomethacin (10 mg/kg i.v.) administered 10 min prior to Sadamin. In contrast, pretreatment with an inhibitor of nitric oxide synthase L-NAME (10 mg/kg i.v.) did not affect to the thrombolytic activity of Sadamin but it significantly inhibited hypotensive action of the drug. (*Table 1, 2*).

Table 1. The influence of indomethacin or L-NAME on thrombolytic action of Sadamin at a dose of 30 mg/kg i.v. in rats.

Drugs	Thrombolysis in %		n
	Before treatment	After treatment	
Indomethacin (10 mg/kg i.v.)	30.7 ± 2.7	22.3 ± 2.4 *	6
L-NAME (10 mg/kg i.v.)	29.5 ± 2.4	27.0 ± 2.8	7

*$p < 0.05$

Experimental data expressed as mean $\pm$ SEM. for (n) number of experiments $p < 0.05$ were considered as significant.

Table 2. The influence of indomethacin or L-NAME on hypotensive action of Sadamin at a dose of 30 mg/kg in rats.

Drugs	Lowering of BP in mm Hg			n
	Control BP	Before treatment	After treatment	
Indomethacin (10 mg/kg i.v.)	110 ± 3.6	21.7 ± 4.8	20.8 ± 4.9	6
L-NAME (10 mg/kg i.v.)	105 ± 7.2	25.0 ± 2.4	17.1 ± 2.6 *	7

*$p < 0.05$

Experimental data expressed as nean $\pm$ SEM. for (n) number of experiments $p < 0.05$ were considered as significant.

ADP-induced platelet aggregation in PAOD patients

Sadamin at a dose of 10 mg/kg i.v. in PAOD patients produced antiplatelet effect expressed as decreasing of the susceptiblity of blood platelets to ADP. After 3 h the infusion of Sadamin on the 1 st, 10 th and 21 st day of the therapy the threshold proaggregatory concentration of ADP (expressed as EC_{30} of ADP-induced platelet aggregation) increase 4.5 ± 0.7 vs. 3.5 ± 0.8 μM, 5.9 ± 0.9 vs 3.8 ± 0.6 μM and 5.6 ± 1.0 vs. 3.6 ± 0.8 μM respectively. (*Fig.1*).
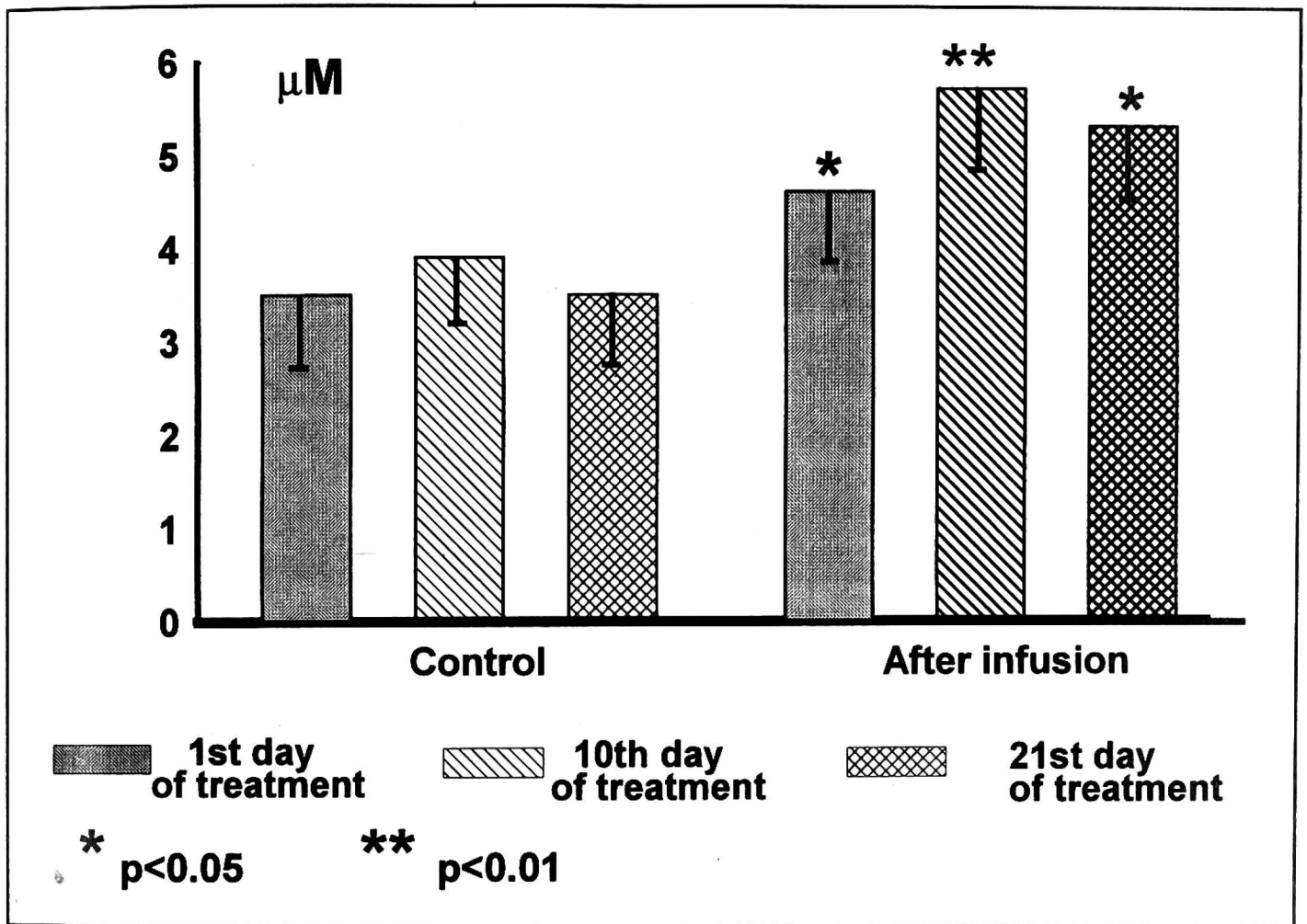
Fig. 1. The influence of infusions of Sadamin (10mg/kg/3 h) on threshold proaggregatory concentrations of ADP (EC_{30}) in PAOD patients, mean $\pm$ S.E.M., n = 20.

Spontaneous platelet aggregability

In PAOD patients the infusion of Sadamin inhibited significantly spontaneous platelet aggregability increasing PAR 0.72 ± 0.05 vs. 0.56 ± 0.07 on 1 st day, 0.77 ± 0.07 vs. 0.6 ± 0.06 on 10 th and 0.85 ± 0.09 vs 0.69 ± 0.09 on 21 st day of the therapy. PAR increased after the 1st infusion of Sadamin and its slightly increased level persisted for all the time of the treatment. (*Fig 2*).

Fibrinolytic action in PAOD patients: euglobulin clot lysis time (ECLT)

Administration of Sadamin at a dose of 10 mg/kg i.v. in PAOD patients produced the fibrinolytic effect expressed as shortening ECLT after
every infusion of Sadamin on 1 st, 10 th and 21 st day of the therapy.
(105 ± 15 vs. 160 ± 20 min, 102 ± 10 vs. 155 ± 15 min and 80 ± 10 vs 140 ± 14 min respectively).
ECLT decreased after the 1st infusion and its slightly decreased level persisted for all the time of the treatment. (*Fig. 3*).
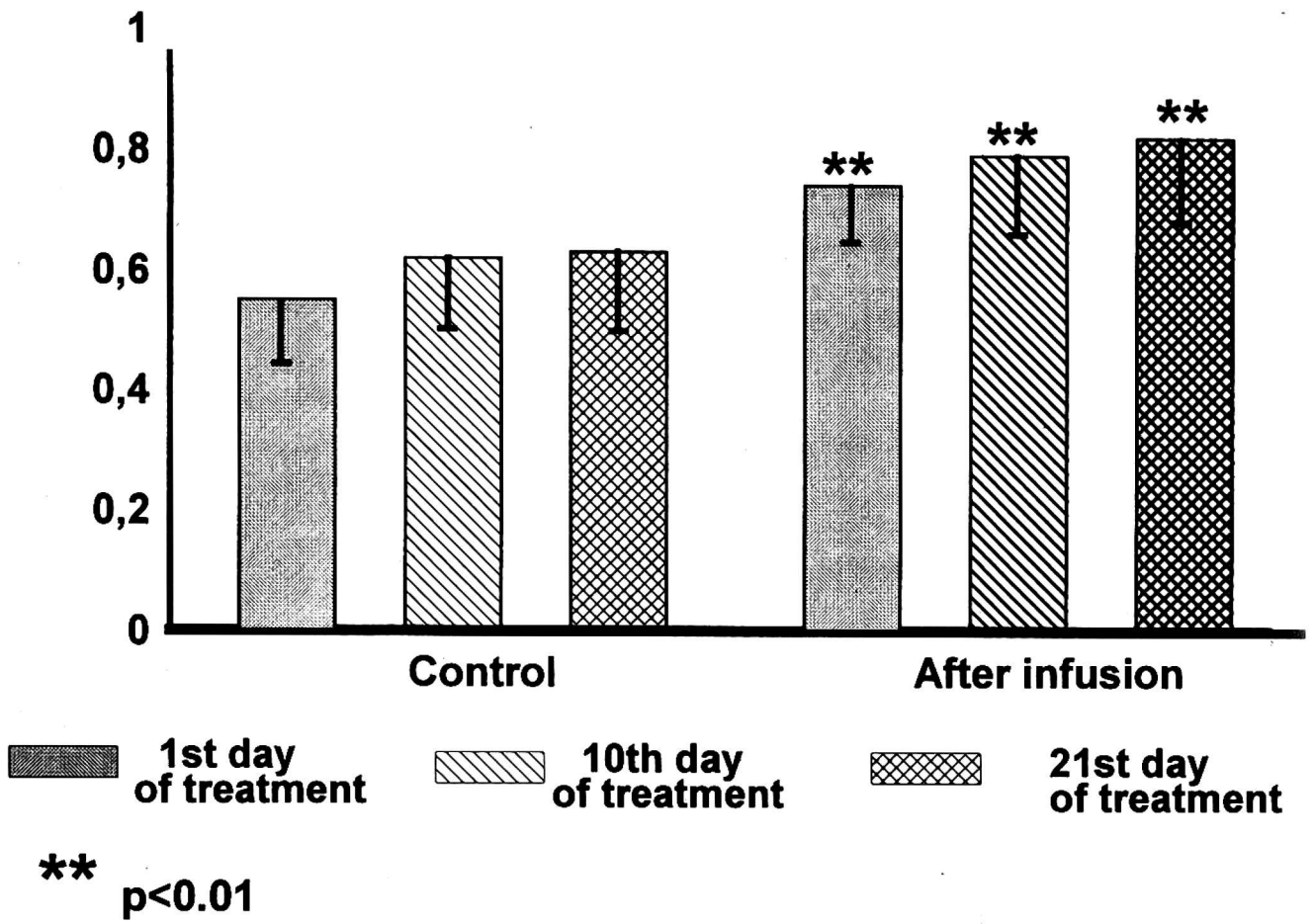
Fig. 2. The influence of infusions of Sadamin (10 mg/kg/3 h) on platelet aggregates ratio (PAR) in PAOD patients, mean ± S.E.M., n = 20.

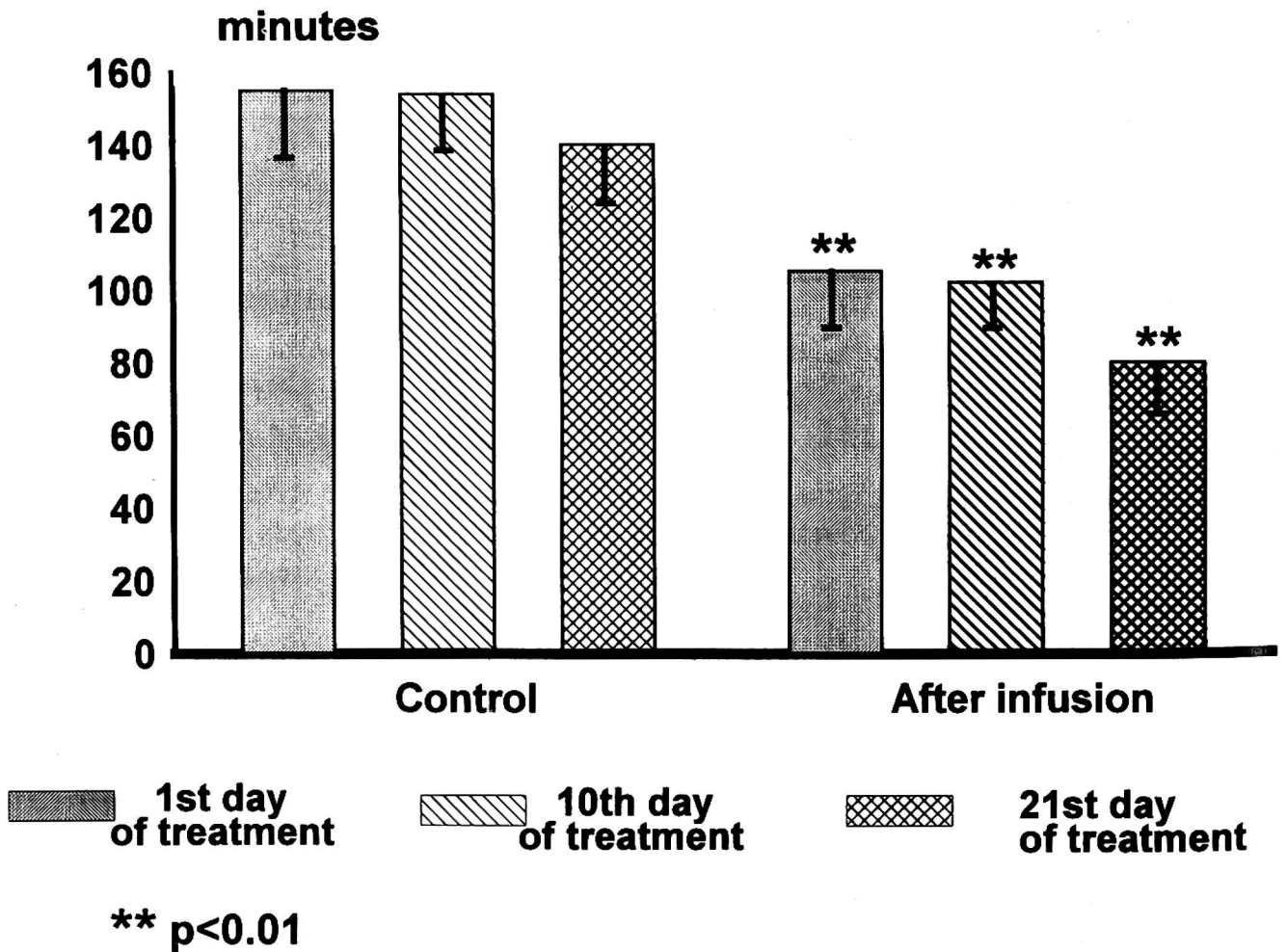*Fig. 3.* The influence of infusions of Sadamin (10 mg/kg/3 h) on euglobulin clot lysis time (ECLT) in PAOD patients, mean ± S.E.M, n = 20.

DISCUSSION

Here we report that Sadamin is not only a vasorelaxant but also fibrinolytic and anti-platelet agent in PAOD patients, moreover, it exerts thrombolytic action in rats. In rats Sadamin-induced thrombolysis was selectively put down by the pretreatment with indomethacin that inhibited cyclooxygenase-1 in endothelial cells, whereas the pretreatment with L-NAME (inhibition of NO synthase) suppressed only hypotensive response to Sadamin. Therefore, it may be submitted that in rats Sadamin exerts its thrombolytic action through endothelial PGI_2 while its hypotensive action is mediated by endothelial NO. Indeed, we have shown that in response to various stimuli PGI_2 and NO are released from endothelial cells in a coupled manner (12—16) but they do not necesserily interact with each other. PGI_2 seems to be mainly responsible for anti-platelet, anti-thrombotic and thrombolytic effects, while NO predominantly for endothelium-mediated vasodilatation. (17—21).

In PAOD patients Sadamin at a dose of 10 mg/kg i.v. inhibited spontaneous and ADP-induced platelet aggregation, and activated plasma fibrinolytic system as evidenced by shortening of ECLT and lowered arterial blood pressure. We have no reason to propose different mechanisms for these actions of Sadamin in PAOD patients than those described here in animal experiments. We propose that platelet suppression and activation of fibrinolysis by Sadamin in PAOD patients are mediated by endothelial PGI_2 while hypotensive effect of Sadamin is NO-mediated.

Fifteen years ago (22) we tried in PAOD patients another nicotinate derivative-ß-pyridilcarbinol (Ronicol) for its fibrinolytic and anti-platelet actions. Then we reported that these pharmacological properties of Ronicol disappeared after pretreatment with aspirin at a high dose of 1.5 g. Furthermore, Ronicol released a PGI_2-like substance into arterial blood of PAOD patients and this release was blocked by aspirin (3). Those days NO was not yet known to be a partner of PGI_2 in endothelium. Even so, our data of Ronicol (3, 22) together with present reports on Sadamin strongly indicate that drugs which accomodate a nicotinate moiety exert their anti-platelet and fibrinolytic actions mainly through the release of endothelial PGI_2. Presently, we may add that their hypotensive action is associated with endothelial release of NO. In case of Sadamin these endothelium-mediated effects are enhanced by the presence of a theophylline derivative, that inhibits decomposition of the second messengers for PGI_2 or NO, i.e. cyclic-AMP or cyclic-GMP, respectively (4, 5).

On the other hand, one cannot exclude decisively synergistic anti-platelet and fibrinolytic actions of endogenous PGI_2 and NO which are being released simultaneously by Sadamin. We reported on a such synergism between exogenous PGI_2 and molsidomine (an NO-donor) in PAOD patients (23, 24).

This finding may not be pertinent to a situation when endogenous PGI_2 and NO are released by Sadamin. Actually, our present experiments in rats speak against such a possibility.

In summary, Sadamin shows hypotensive, anti-platelet and fibrinolytic properties in PAOD patients and vasorelaxant and thrombolytic actions in rats. Our data point to a possibility that vasorelaxant properties of Sadamin are mediated by endothelial NO while its anti-platelet/thrombolytic properties depend on the endothelial release of PGI_2. It is likely that both endothelial mediators are released by the nicotinate moiety of Sadamin while its theophylline part is responsible for reinforcement of action of endogenous PGI_2 and NO at the level of their second messengers, i.e. c-AMP and c-GMP, respectively.

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Author's address: Krzysztof Bieroń, Chair of Pharmacology, Jagiellonian University Medical College, ul. Grzegórzecka 16, 31-351 Kraków, Poland.
tel. +(48 12) 421-10-60 fax +(48 12) 421-72-17