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ANTITHROMBOTIC EFFECT OF L-ARGININE IN HYPERTENSIVE RATS

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The aim of the study was to evaluate the effect of L-arginine (L-Arg) on haemostasis in stasis model of venous thrombosis in renal hypertensive rats. The effect of the single dose (i.v.300 mg/kg bolus+300 mg/kg/h) and of the 10-day application (p.o. 1 g/kg, once daily) of L-Arg was determined. L-Arg reduced the blood pressure both in the acute and long-term application. The single dose of L-Arg decreased the occurrence rate of the thrombus whereas long-term administration reduced significantly the thrombus weight. There were no differences in prothrombin time and activated partial thromboplastin time while the fibrinogen concentration decreased both in the acute and the long-term experiment. L-Arg shortened euglobulin clot lysis time and bleeding time in the long-term application. The chronic L-Arg treatment also inhibited significantly collagen-induced platelet aggregation. The overall haemostasis and coagulation potentials were inhibited and the fibrinolysis potential was higher in the group receiving this amino-acid. The results show that L-Arg, in a complex way, evokes the antithrombotic effect in the model of venous thrombosis in hypertensive rats.

Key words: *L-arginine, haemostasis, venous thrombosis, rats*

INTRODUCTION

The vascular endothelium produces a large number of biological substances, of which nitric oxide (NO) is the best known. NO originates in a complex reaction of L-Arg oxidation, in which also L-citrulline is produced (1-3).

Nitric oxide has been shown to be an extremely important signaling molecule in cardiovascular system (3,4). Although, in the last decade many attempts were done to prevent cardiovascular disease by changing life style and diet, sufficient therapies have not been found yet. Thus, the possibility to increase nitric oxide

availability and to enhance the enzymatic activity of NO synthase by the simultaneous treatment with L-Arg has been postulated. Both *in vitro* (5,6) and *in vivo* (7-9) studies have demonstrated that L-Arg as a precursor for nitric oxide can augment vascular dilation under certain conditions. According to these findings, it was shown that a long-term administration of L-Arg ameliorated peripheral (mainly muscle) insulin sensitivity through a normalization of NO/cGMP pathway in lean type 2 diabetic patients (10) and that an increased NO availability, after an acute infusion of L-Arg, significantly improved forearm oxidative glucose metabolism in subjects with microvascular angina (11).

Besides blood pressure, the majority of literature reports refer to the interaction of L-Arg with blood platelets (9,12-14). L-Arg via enhancement of nitric oxide synthesis in endothelium activates intraplatelet guanylate cyclase, thus increasing cGMP concentration, and prevents platelet adhesion and aggregation (15-17).

Besides that, up to now, only few reports have appeared concerning L-Arg and haemostasis. Haemostasis is a complex processes that involves: vascular wall, blood platelets, coagulation and fibrinolytic system interactions. Stief et al. have observed a prolongation of PT and APTT after L-Arg incubation with human plasma (18). It has been also shown that L-Arg inhibits PAI release from platelets and stimulates the activity of endothelium-derived t-PA (13).

Taking the above data into account as well as the fact that L-Arg is the only substrate for NO synthesis, it can be assumed that this amino acid may also modify coagulation and fibrinolysis *in vivo*. The present study is an attempt to find the potential antithrombotic action of L-Arg, including the effect of L-Arg on primary haemostasis, coagulation and fibrinolysis in the model of venous thrombosis in hypertensive rats.

MATERIAL AND METHODS

Animals and renovascular hypertension induction

Male Wistar rats (200g) were used in the experiments. Two kidney, one clip model of hypertension was induced by partial, standardized clipping of the left renal artery under pentobarbital anesthesia (45 mg/kg, i.p.) (19). After 6 weeks all the animals developed hypertension which was confirmed by the blood pressure measurement using "tail cuff" method (20). The animals were housed in a room with a 12h light/dark cycle, in group cages as appropriate, were given tap water and fed a standard rat chow. Twenty four hours before the induction of venous thrombosis, the rats were deprived of food but had free access to water. Only animals with blood pressure higher than 140 mm Hg were included in the experiments. Procedures involving the animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and Guidelines for the Use of Animals in Biomedical Research (Thromb Haemost 1987; 58: 1078-84).

Drugs and reagents

L-arginine (L-arginine Hydrochloride, Sigma Chemical Co., USA), pentobarbital (Vetbutal, Biovet, Poland), buffer Tris [Tris(hydroxymethyl)-aminomethane hydrochloride (Sigma Chemical Co., USA) and Tris(hydroxymethyl)-aminomethane (Merck, Germany)], collagen (Chronolog, USA), trisodium citrate (Polish Chemical Reagents), calcium chloride (CaCl2) (Polish Reagents Chemical) were used in the experiments.

L-arginine administration

In the acute experiment, rats received a single dose of L-Arg by intravenous infusion (300 mg/kg bolus + 300 mg/kg/h for 2h). Control rats were given physiological saline in the same way and in the same volume. In the chronic experiment, L-Arg, dissolved in distilled water was administered by intragastric probe always at the same time for 10 days (1g/kg, once daily). Control group received distilled water also per os, at the same time and in the same volume as the L-Arg group.

Venous thrombosis

The animals were anaesthetized with pentobarbital (45mg/kg, i.p.). Venous thrombosis was performed as previously described by Reyers et al.(21). Briefly, the abdomen was opened, the vena cava was carefully separated from surrounding tissues and then ligated tightly with a cotton thread just below the left renal vein. Subsequently, the abdomen was closed with a double layer of sutures (peritoneum with muscles and the skin separately). After two hours the animals were re-anaesthetized and the abdomen was then reopened, the vena cava was carefully dissected and inspected for the presence of thrombus. The thrombus was kept at 37°C and after 24 hours its dry weight was measured.

Direct blood pressure measurement

Mean blood pressure in rats receiving L-Arg infusion was measured directly through a cannula filled with heparin solution, placed in the right common carotid artery and connected to a pressure transducer (PD-23 Gould) and monitor Trendoscope 8031, Unitra Biazet S&W Medico Teknik, A/S Denmark).

Bleeding time (BT)

"Template" bleeding time was measured just before venous thrombosis induction by longitudinal incision of a rat tail according to Dejana et al. (22)

Haemostatic parameters (PT, APTT, ECLT and fibrinogen concentration)

Blood samples were taken from the heart two hours after venous thrombosis induction. All samples were mixed with 3.13% trisodium citrate in a volume ratio 10:1. Prothrombin time (PT), activated partial thromboplastin time (APTT), euglobulin clot lysis time (ECLT) and fibrinogen concentration were determined by routine laboratory assays.

Whole blood platelet aggregation

The blood was collected and added to 0.9% NaCl (1:1), poured to cuvettes (1 ml in each) and incubated for 15 minutes at 37°C. Then, it was mixed for 1 minute and collagen (5µg/ml) was

added. Platelet aggregation was measured for 6 minutes according to the method of Cardinal (23) using a Lumi-aggregometer (Chrono-log, USA). A change in blood resistance was expressed in ohms.

Haemostatic potentials (HP)

The haemostatic potential was assessed according to the method of He S et al. (24,25). From pentobarbital-anaesthetized (45 mg/kg) control and drug-receiving rats blood was collected, mixed with trisodium citrate (10:1) and centrifuged at 2000 x g for 20 minutes at 4°C. In the plasma so prepared, the haemostatic potentials (HP) were determined or the 0.5 ml plasma samples were frozen at -70°C and defrosted just before HP assay.

a) Assessment of the overall coagulation potential (OCP)

120 µl of plasma was poured to the first rows of microplatelets. 100 µl of 66 mM buffer Tris, pH 7.4, as well as thrombin, $CaCl_2$ and NaCl (final concentrations: 0.005 IU/ml, 17 mM and 65 mM, respectively) were added to each sample. Referential samples (blank) contained only 120µl of plasma and 100 µl of buffer Tris. The mixtures, 4 minutes after the beginning of buffer addition, were placed in a microplatelet reader (Dynex Technologies Revelation II, USA) and fibrinous thrombus growth was measured every minute for 15 minutes at 405 nm wavelength. The OCP was evaluated as sum of the increment in optical density of the tested sample compared to the referential sample (blank).

b) Assessment of the overall haemostatic potential (OHP)

The OHP was determined at the same time and in the identical way as the OCP, using the same platelet (further rows), but to each plasma sample, buffer Tris was added with composition as for the OCP and additionally containing t-PA at the final concentration of 1500 ng/ml.

c) Assessment of the overall fibrinolytic potential (OFP)

The OFP was expressed in percentages and showed the difference between the OCP and OHP, which was demonstrated by the difference between OCP and OHP calculated using the formula:

$$OFP = OCP\text{-}OHP / OCP \times 100\%.$$

Statistical analysis

Multiple group comparisons were performed by one-way analysis of variance (ANOVA), and when significant intergroup differences occurred, were assessed by a Student-Neuman-Keuls test. Incidence of venous thrombosis was evaluated by Fisher's exact test. Student's unpaired t-test was also used to determine the significance between means for two groups. A value of $p<0.05$ was considered statistically significant.

RESULTS

The mean blood pressure of anaesthetized rats was 158 ± 13.12 mmHg. Infusion of physiological saline for 300 seconds had no effect on this parameter (*Fig.1A*). L-Arg caused a sudden pressure drop between 40 and 150 second, with the maximum effect at 60 second (drop by 49 mm Hg). At 210 second the mean pressure returned to the initial level. The pressure reduction between 40 and 150 second was statistically significant ($p<0.05$). Further infusion (for two hours) of physiological saline or L-Arg did not change the blood pressure (data not shown). In the animals

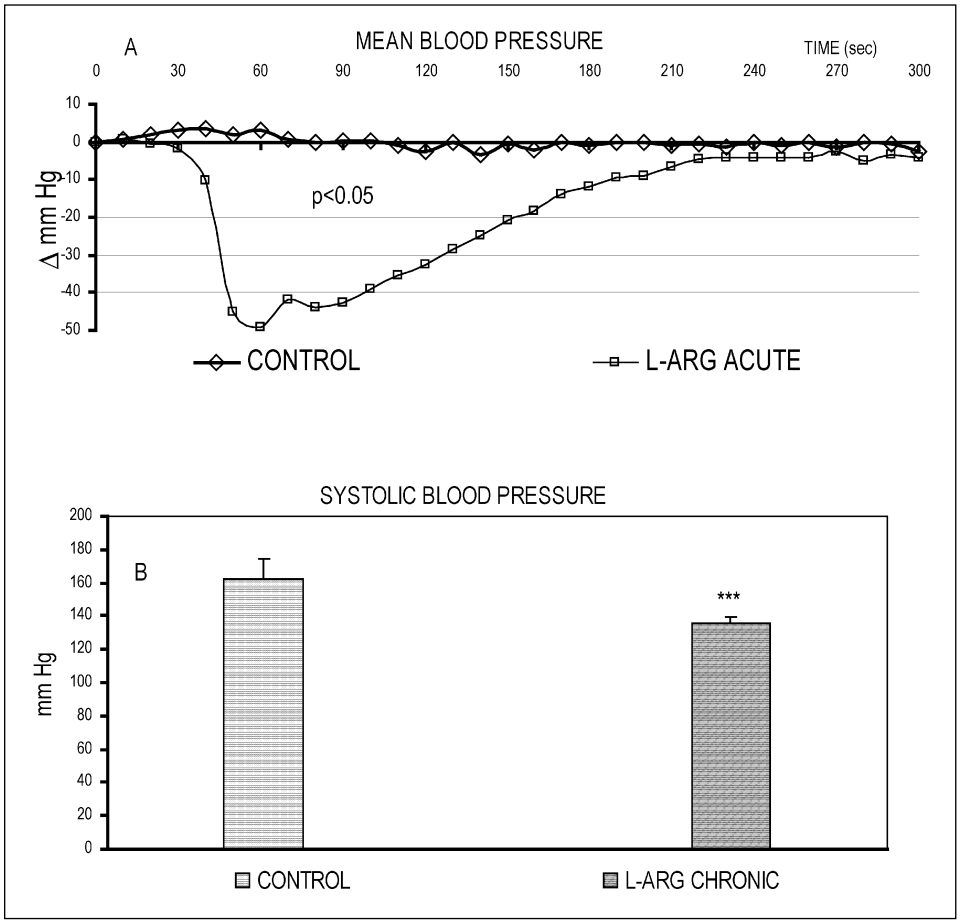

Fig. 1. Graph represents (A) the mean blood pressure in rats acutely treated with L-arg (300 mg/kg bolus and 300 mg/kg/h i.v. for 2 hours, n=8) in comparison to the control (statistical significance between 40 and 150 second: p<0,05 vs control) and columns (B) represent the systolic blood pressure in rats chronically treated with L-arg (1g/kg p.o., once daily, for 10 days, n=10) in comparison to control (***p<0.001 vs control). Data are expressed as means ±SD.

receiving L-Arg per os, the systolic pressure decreased to 135.8 ± 3.29 mm Hg, in comparison to control group being 162 ± 12.74 mmHg (p<0.001) (*Fig.1B*).

Two hours after experimental induction of venous thrombosis, thrombus was found in all control and L-Arg chronically treated animals, whereas only 75% of rats receiving L-Arg intravenously were occluded (p<0.01). The mean dry weight of thrombus was slightly reduced in animals given L-Arg, but the difference was statistically insignificant. In chronic experiment, thrombus was found in all control rats, but the mean dry mass of venous thrombus in L-Arg treated group was markedly reduced from 4.98 ± 1.56 mg to 1.56 ± 0.73 mg (p<0.001) (*Fig.2*).

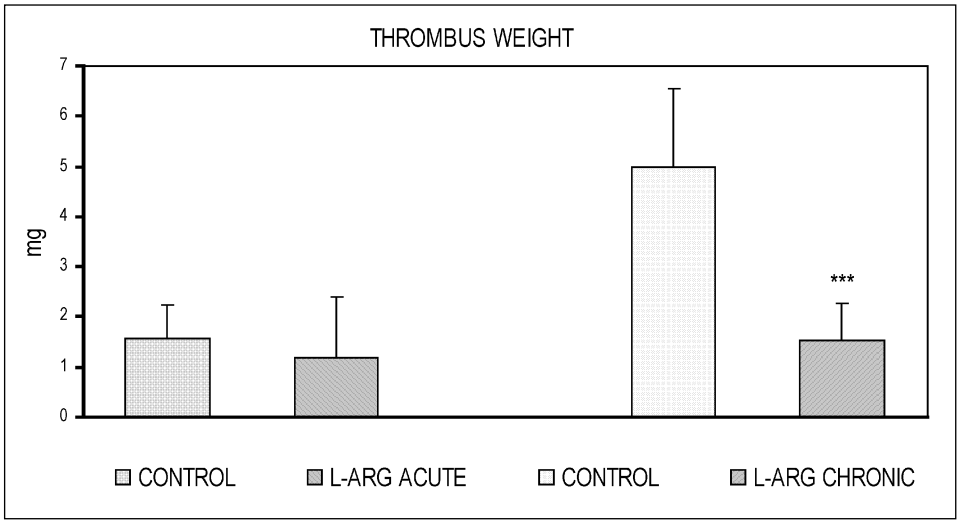

Fig. 2. Columns represent thrombus weight in rats acutely treated with L-arg (300 mg/kg bolus and 300 mg/kg/h i.v. for 2 hours, n=8) in comparison to control and chronically treated with L-arg (1g/kg p.o. for 10 days, n=10) in comparison to control [***p<0.001 vs control]. Data are expressed as means ±SD.

The PT and APTT were not changed by L-Arg administration both in acute and chronic experiments (*Table I*). Intravenous administration of L-Arg caused a decrease in fibrinogen concentration from 266.6 ± 36.3 mg/dl (control group) to

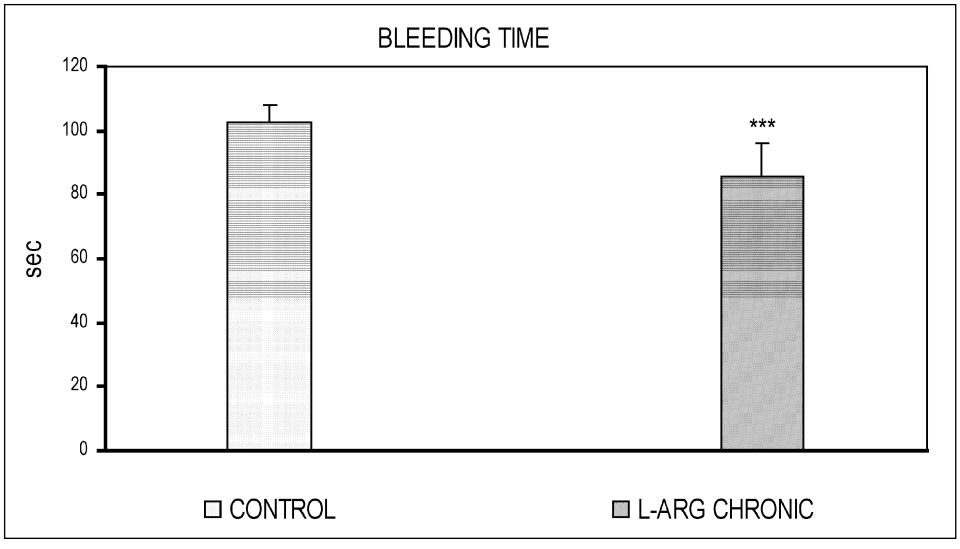

Fig. 3. Columns represent the "template" bleeding time in rats chronically treated with L-arg (1g/kg p.o. for 10 days, n=10) in comparison to control (***p<0.001 vs control). Data are expressed as means ±SD.

Table I. Activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen concentration and euglobulin clot lysis time (ECLT) in rats treated with L-arginine in acute (300mg/kg bolus and 300mg/kg/h i.v. for 2 hours, n=8) or chronic (1g/kg p.o., for 10 days, n=10) manner in comparison to control. Data are expressed as means±SD. *p<0.05, ***p<0.001 vs control

	PT(s)	**APTT(s)**	**Fibrinogen concentration (mg/dl)**	**ECLT (minutes)**
CONTROL ACUTE i.v.	34,17±2,76	18,51±0,46	266,6±36,3	319,75±52,22
L-ARG ACUTE i.v.	34,59±1,37	18,17±0,1	227,5±23,5*	297,5±35,35
CONTROL CHRONIC p.o.	25,87±2,43	18,82±1,17	454,2±114,2	393,0±128,41
L-ARG CHRONIC p.o.	27,07±3,53	18,43±0,3	267,0±27,4***	287,0±29,0*

227.5 ± 23.5 mg/dl (p<0.05). Oral administration of the drug led to a statistically significant decrease in this parameter from 454.2 ± 114.2 mg/dl (control rats) to 267.0 ± 27.4 mg/dl (p<0.001). The ECLT after intravenous and oral L-Arg administration was shortened, although the difference was statistically significant only in chronic experiment (p<0.05). In chronic experiment the BT was 102.3 ±

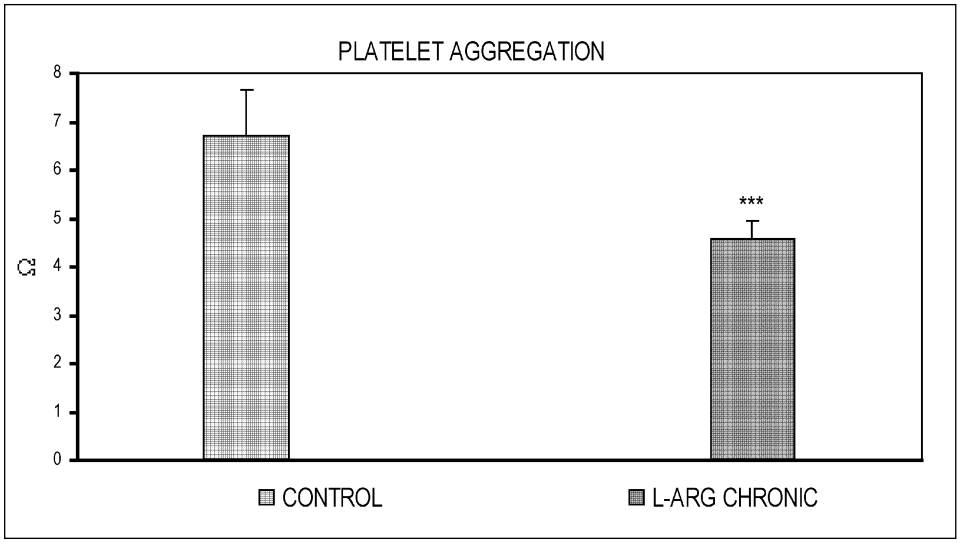

Fig. 4. Columns represent the collagen-induced platelet aggregation in rats chronically treated with L-arg (1g/kg p.o. for 10 days, n=10) in comparison to control (***p<0.001 vs control). Data are expressed as means ±SD.

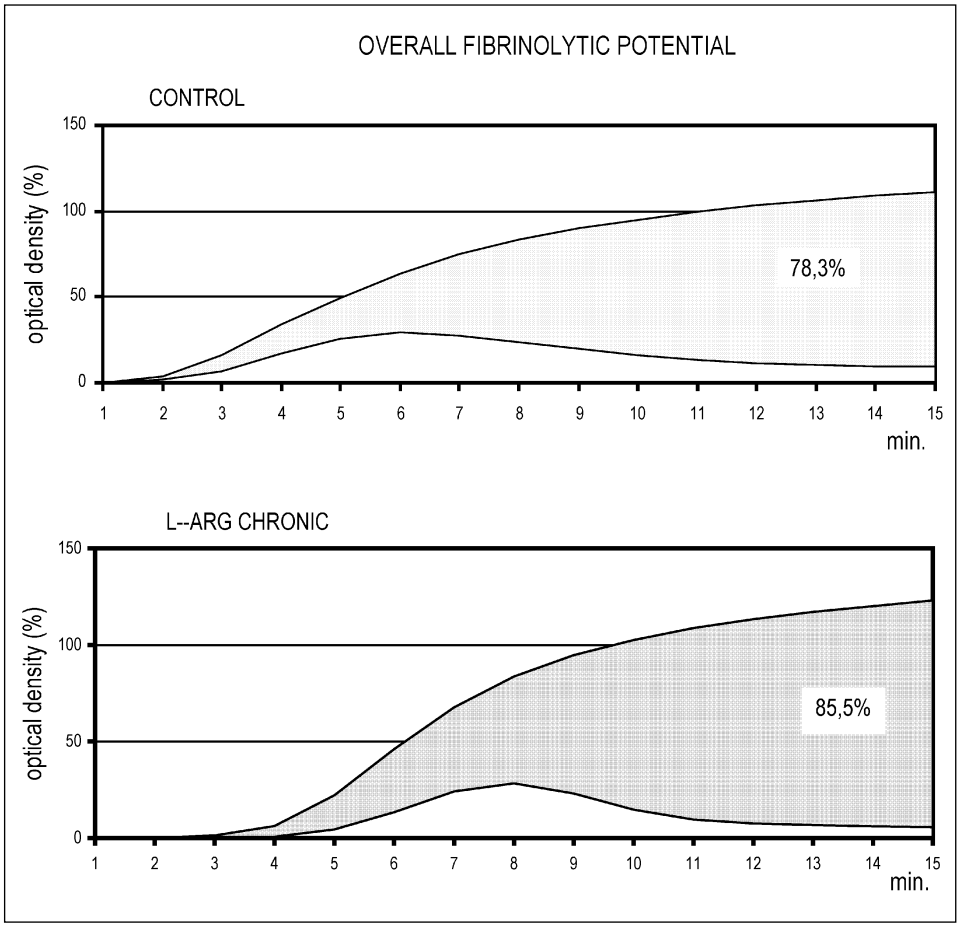

Fig. 5. Graphs represent the overall fibrynolytic potential in rats chronically treated with L-arg (1g/kg p.o. for 10 days, n=10) in comparison to control ($p<0,05$ vs control). Data are expressed as means ±SD.

5.8 sec in control animals and it was shortened to 85.6 ± 10.6 sec. in the L-Arg treated group ($p<0.001$) (*Fig.3*).

Collagen-induced platelets aggregation expressed as impedance measured between electrodes embedded in a whole blood sample was 6.72 ± 0.93 Ω in control group (*Fig.4*). In the L-Arg chronic treated group (p.o.) this value was significantly reduced to 4.56 ± 0.93 ‘Ω ($p<0.001$).

The overall coagulation potential was presented as a sum of optical density increments in a 15 min period at 405 nm wavelength in relation to blind test, being respectively: 1040.1 (control) and 1006.8 (L-Arg chronic treated group). The overall haemostatic potential was analogically calculated and was 223.6 in control group and 143.8 in L-Arg group. The overall fibrinolytic potential in

control group was 78.3% and in L-Arg group - 85.5% of optical density (*Fig.5*). This change was statistically significant ($p<0.05$).

DISCUSSION

The aim of the present study was to evaluate the effect of L-Arg on the experimental venous thrombosis in rats. The study employed a simple and easy to perform model of venous thrombosis according to Reyers et al. (21). It is not only simple but also sensitive to study the action of haemostasis-affecting agents (19,26,27). The process of thrombus formation in this model is largely NO and PGI_2 dependent (19,26,27). In our study L-Arg administered per os for 10 days considerably suppressed the formation of venous thrombus in rats. Such characteristic changes were not observed when a single intravenous dose of L-Arg was used. The assessment of L-Arg involvement in haemostasis should take into consideration its effect on blood vessels, platelets, coagulation and fibrinolytic systems. Since L-Arg was used in doses possessing hypotensive effect, one might consider that its antithrombotic activity is a result of the reduction in blood pressure.

Indeed, our findings indicate that L-Arg administered i.v. in a single dose as well as chronically p.o. reduced arterial blood pressure. During intravenous infusion the pressure returned to the initial level already at 210 second and remained unchanged during two hours of observation. Thus, at the time of venous thrombosis induction (after two hours of L-Arg infusion) we did not observe any differences in BP between the control animals and the rats treated intravenously with L-Arg. Therefore, the contribution of a hypotensive effect in the antithrombotic effect of the tested amino acid should be excluded. This is in accordance with the findings of previous experiments where no correlation between hypotensive action of some drugs and antithrombotic effect was found (26,27).

Apart from vessels, blood platelets play a very important role in primary haemostasis. The literature provides well documented and defined antiaggregatory effect of L-Arg not only in whole blood but also in platelet-rich plasma. The aggregation has been induced by ADP (7,9,12,13,18), collagen (28) or thrombin (29). Our findings confirm that L-Arg suppresses platelet aggregation in whole blood. Other authors also demonstrated that this effect is associated with enhanced NO synthesis (15-17,30,31).

In the regulation of primary haemostasis, the interaction of blood vessel with platelets plays an essential role and its *in vivo* evaluation involves bleeding time measurement. We found a significant shortening and not prolongation as expected of the bleeding time after 10-day L-Arg administration. A similar tendency was observed by Gruszecki et al. (32) during administration of propranolol. This may be associated with a considerable reduction in arterial blood pressure caused by

administration of drugs, being the result of a direct relaxatory effect on vascular smooth muscle cells.

Secondary haemostasis is another extremely important factor determining thrombus formation in vessels. Blood clotting can be inhibited by e.g. attenuation of plasma prothrombin activation system. Only few reports have appeared on the effect of L-Arg on the plasma clotting component. Stief et al. (18) exposed human plasma to the action of various concentrations of L-Arg *in vitro*, showing that the increasing amounts of this amino acid proportionally extend the prothrombin and activated partial thromboplastin time. Therefore, the influence of L-Arg on certain factors of the clotting system was examined. No significant differences were observed in the PT and APTT after i.v. and p.o. administration. However, fibrinogen concentration, especially in chronic administration of L-Arg was decreased, which was also confirmed by other authors (33).

The effect of L-Arg on the fibrinolytic system cannot be omitted here. We found that ECLT was considerably shortened, but only after 10-day L-Arg administration. We also noted the increased overall fibrinolytic potential. These results are in accordance with the data obtained by Gryglewski et al. (13) and Dambisya et al. (34).

Concluding, L-Arg administered chronically exerts an antithrombotic effect, which is associated with the suppression of platelet aggregation and increased fibrinolytic activity. This action seems to be indirect and occur *via* NO synthesis.

Currently, L-Arg is used in the treatment of liver failure, where due to stimulation of the urea cycle it removes toxic ammonia from the organism. This amino acid is also used to prevent vascular disease proceeding particularly with endothelium impairment (10,11,35). The results of our study may contribute to the extension of L-Arg indication also to antithrombotic therapy or to the prophylaxis of thromboembolic disorders accompanying many systemic disorders in humans.

REFERENCES

1. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; 327: 524-526.
2. Palmer RM. The L-arginine:nitric oxide pathway. *Curr Opin Nephrol Hypertens* 1993; 2: 122-128.
3. Loscalzo J. What we know and don't know about L-arginine and NO. *Circulation* 2000; 101: 2126-2129.
4. Moncada S. Nitric oxide. *J Hypertens* 1994; 12 (Suppl. 10): S35-S39.
5. Böger RH, Bode-Böger SM, Mugge A, Kienke S, Brandes R, Dwenger A et al. Supplementation of hypercholesterolaemic rabbits with L-arginine reduces the vascular release of superoxide anions and restores NO production. *Atherosclerosis* 1995; 117: 273-284.
6. Chen J, Kuhlencordt P, Urano F, Ichinose H, Astern J, Huang PL. Effects of chronic treatment with L-arginine on atherosclerosis in apoE knockout and apoE/inducible NO synthase double-knockout mice. *Arterioscler Tromb Vasc Biol* 2003; 23:97-103.

7. Giugliano D, Marfella R, Verrazzo G, Acampora R, Nappo F, Ziccardi P et al. L-arginine for testing endothelium-dependent vascular functions in health and disease. *Am J Physiol* 1997; 273: E606-E612.
8. Maxwell AJ, Zapien MP, Pearce GL, MacCallum G, Stone PH. Randomized trial of a medical food for the dietary of chronic, stable angina. *J Am Coll Cardiol* 2002; 39:37-45.
9. Bode-Böger SM, Böger RH, Creutzig A, Tsikas D, Gutzki FM, Alexander K et al. L-arginine infusion decreases peripheral arterial resistance and inhibits platelet aggregation in healthy subjects. *Clin Sci* 1994; 87: 303-310.
10. Piatti PM, Fragasso G, Monti LD, Setola E, Lucotti P, Fermo I et al. Acute intravenous L-arginine infision decreases endothelin-1 levels and improves endothelial function in patients with angina pectoris and normal coronary arteriograms. *Circulation* 2003;107: 429-436.
11. Piatti PM, Monti LD, Valsecchi G, Magni F, Setola E, Marchesi F et al. Long-term oral L-arginine administration improves peripheral and hepatic insulin sensivity in type 2 diabetic patients. *Diabetes Care* 2001; 24: 875-880.
12. Adams MR, Forsyth CJ, Jessup W. Robinson J., Celermajer D.S.: Oral L-arginine inhibits platelet aggregation but does not enhance endothelium-dependent dilation in healthly young men. *J Am Coll Cardiol* 1995; 26: 1054-1061.
13. Gryglewski RJ, Grodzińska L, Kostka-Trąbka E, Korbut R, Bieroń K, Goszcz A et al. Treatment with L-arginine is likely to stimulate generation of nitric oxide in patients with peripheral arterial obstructive disease. *Wien Klin Wochenschr* 1996; 108: 111-116.
14. Preli RB, Klein K, Herrington D. Vascular effects of dietary L-arginine supplementation. *Atherosclerosis* 2002;162:1-15.
15. Anfossi G., Russo I., Massucco P., Mattiello L., Perna P., Tassone F., Trovati M.: L-arginine modulates aggregation and intracellular cyclic 3'5'- guanosine monophosphate levels in human platelets: direct effect and interplay with antioxidative thiol agent. *Thromb Res* 1999; 94: 307-316.
16. Bode-Böger SM, Böger RH, Kienke S, Bohme M, Phivthong-ngam L, Tsikas D, Frölich JC. Chronic dietary supplementation with L-arginine inhibits platelet aggregation and thromboxane A2 synthesis in hypercholesterolaemic rabbits in vivo. *Cardiovasc Res* 1998; 37: 756-764.
17. Bode-Böger SM, Böger RH, Galland A, Frölich JC. Differential inhibition of human aggregation and thromboxane A_2 formation by L-arginine in vivo and in vitro. *Arch Pharmacol* 1998; 357: 143-150.
18. Stief TW, Weippert M, Kretschmer V, Renz H. Arginine inhibits hemostasis activation. *Thromb Res* 2001; 104: 265-274.
19. Chabielska E, Pawlak R, Wollny T, Ró łkowski R, Buczko W. Antithrombotic of losartan in two kidney, one clip hypertensive rats. A study on the mechanism of action. *J Physiol Pharmacol* 1999; 50: 99-109.
20. Zatz R.: A low-cost tail-cuff method for the estimation of mean arterial pressure in conscious rats. *Lab Anim Sci* 1990; 42: 198-201.
21. Reyers I, de Gaetano G, Donati MB. Venostasis-induced thrombosis in rat is not influenced by circulating platelet or leukocyte number. *Agents Actions* 1989; 28: 137-140.
22. Dejana E, Callioni A, Quintana A, de Gaetano G. Bleeding time in laboratory animals. II-a comparison of different assay conditions in rats. *Thromb Res* 1979;15:191-197.
23. Cardinal DC, Flower RJ. The electronic aggregometer: a novel device for assessing platelet behavior in blood. *J Pharmacol Methods* 1980; 3: 135-138.
24. He S, Bremme K, Blomback M. A labolatory method for determination of overall haemostatic potential in plasma .I. Method design and preliminary results. *Thromb Res* 1999; 96: 145-156.

25. He S, Antovic A, Blombäck M. A simple and rapid labolatory method for determination of haemostasis potential in plasma.II. Modifications for use in routine laboratories and research work. *Thromb Res* 2001; 103: 355-361.
26. Chabielska E, Pawlak R, Golatowski J, Rółkowski R, Pawlak D, Buczko W. Losartan inhibits experimental venous thrombosis in spontaneously hypertensive rats. *Thromb Res* 1998; 90: 271-278.
27. Pawlak R, Chabielska E, Golatowski J, Azzadin A, Buczko W. Nitric oxide and prostacyclin are involved in antithrombotic action of captopril in venous thrombosis in rats. *Thromb Haemost* 1998; 79: 1208-1212.
28. Wolf A, Zalpour C, Theilmeier G, Wang BY, Ma A, Anderson B et al. Dietary L-arginine supplementation normalizes platelet aggregation in hypercholesterolemic humans. *J Am Coll Cardiol* 1997; 29: 479-485.
29. Mendez JD, Zarzoza E. Inhibition of platelet aggregation by L-arginine and polyamines in alloxan treated rats. *Biochem Mol Biol Int* 1997; 43: 311-318.
30. Marietta M, Facchinetti F, Neri I, Piccinini F, Volpe A, Torelli G. L-arginine infusion decreases platelet aggregation through an intraplatelet nitric oxide release. *Thromb Res* 1997; 88: 229-235.
31. Tsao PS, Theilmeier G, Singer AH, Leung LLK, Cooke JP. L-arginine attenuates platelet reactivity in hypercholesterolemic rabbits. *Arterioscler Thromb* 1994; 14: 1529-1533.
32. Gruszecki M, Rółkowski R, Pawlak R, Buczko W. Propranolol prevents the development of venosus thrombosis in rats by a platelet-dependent mechanism. *Pol J Pharmacol* 2001; 53: 5-10.
33. Kawabata A, Hata T. Attenuation by prolonged nitric oxide synthase inhibition of the enhancement of fibrinolysis caused by environmental stress in the rat. *Br J Pharmacol* 1996; 119: 346-350.
34. Dambisya YM, Lee TL. A thromboelastography study on the *in vitro* effects of L-arginine and L- N^G-nitroarginine methyl ester on human whole blood coagulation and fibrinolysis. *Blood Coagulation and Fibrinolysis* 1996; 7: 678-683.
35. Blum A, Hathaway L, Mincemoyer R, Schenke WH, Kirby M, Csako G et al. Oral L-arginine in patients with coronary artery disease on medical management. *Circulation* 2000; 101: 2160-2164.

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