

Effect of captopril and enalapril on selected coagulation markers in hyperlipidemia

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الخلاصة:

إن هدف هذه الدراسة هو تقييم تأثير عقاري (كابتوبريل ، اينالبريل) على عملية تخثر الدم لدى الجرذان ذات مستوى الشحوم المرتفع ثمانية وعشرون ذكرا من جرذان نوع- (Sprague Dawely) أدخلت في هذه الدراسة حيث قسمت بشكل عشوائي إلى أربع مجاميع . (سبعة جرذان في كل مجموعة) .المجموعة الأولى أعطيت غذاء الجرذان الطبيعي واعتبرت مجموعة سيطرة. إما المجموعة الثانية والثالثة والرابعة أعطيت غذاء عالي الدهون لمدة ثمانية أسابيع. المجموعة الثانية أعطيت ماء مقطر واعتبرت مجموعة سيطرة عالية الدهون في حين إن المجموعة الثالثة اعطيت عقار كابتوبريل 25×2 ملم / كم / يوم والمجموعة الرابعة أعطيت عقار انالبريل 15 ملم/كم/يوم لمدة أربع أسابيع تضمنت الدراسة قياس مستوى الكلسترول الكلي وبعض مؤشرات عملية تخثر الدم وهي :- الفايبرينوجين ، بي تي ، أي بي تي وعدد الصفائح الدموية وكانت النتائج كالآتي :- زيادة معنوية ($p < 0.05$) في مستوى الفايبرينوجين في بلازما الجرذان ذات مستوى الشحوم المرتفع بالمقارنة مع مجموعة السيطرة بسبب عقاري الكابتوبريل ، والانالبريل انخفاضاً معنوياً ($p < 0.05$) في مستوى الفايبرينوجين وزيادة معنوية في بي تي و أي بي تي في الجرذان ذات مستوى الشحوم المرتفع ولكن لا يظهر أي تأثيراً احصائياً معنوياً ($p > 0.05$) على عدد الصفائح الدموية. نستنتج ان هذين العقارين يؤثران تأثيراً ملحوظاً وإيجابياً" على عملية تخثر الدم

Abstract:

Background: In addition to their primary indications, hypertension and congestive heart failure, angiotensin converting enzyme inhibitors, ACE-Is, have pleiotropic effects, which are under active investigations. There is growing evidence of data stating the significant role of ACE-Is in the regulation of hemostasis.

Objectives: The present study aimed to evaluate the effect of ACE-Is on coagulation in hyperlipidemia.

Materials and Methods: Twenty-eight male Sprague-Dawely rats aged 17-19 weeks were enrolled in this study. The animals were randomized into four groups each containing seven rats: group 1, 2, 3 and 4. Group 1 was fed a

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standard chow diet and serve as normal diet control while group 2,3 and 4 received atherogenic diet (4% cholesterol-enriched diet) for eight weeks. Animals in group 2, 3 and 4 were treated with distilled water (lipidemic control), captopril and enalapril for the next four weeks respectively. Captopril was used in a dose of 2x25 mg/kg/day P.O. Enalapril was given in a dose of 15 mg /kg/day P.O. At the end of the experiment, the animals were

sacrificed and blood sample was obtained from the heart for determination of prothrombin time (PT), activated partial thromboplastin time (aPTT), plasma fibrinogen, platelets count and total serum cholesterol (TSC).

Results: Hyperlipidemia is associated with significant rise in plasma fibrinogen level ($p < 0.05$). Captopril and enalapril treatment showed significant fall in elevated plasma fibrinogen levels ($p < 0.05$). Hyperlipidemic rats treated with captopril and enalapril for 4 weeks showed significant prolongation of PT and aPTT ($p < 0.05$) while the platelets counts were statistically unaffected ($p > 0.05$).

Conclusion: We conclude that captopril and enalapril possess favorable effect on coagulation in hyperlipidemia.

Key words: captopril, enalapril, hyperlipidemia, coagulation.

Introduction:

Renin-angiotensin system (RAS) consists of several components that constitute one efficiently operating biochemical system responsible for blood pressure regulation and hydroelectrolite balance⁽¹⁾. Angiotensin-converting enzyme inhibitors (ACE-Is) like captopril, enalapril, lisinopril and ramipril produced a significant progress in the treatment of hypertension and congestive heart failure. ACE-Is interfere with the conversion of angiotensin I to angiotensin II, the most powerful vasoconstrictor in the body, leading to decrease angiotensin II level. Angiotensin II acts on two G-protein coupled receptors, which account for all classic actions of angiotensin; vasoconstriction and stimulation of aldosterone production by adrenal cortex. Bradykinin is also a substrate for ACE and contributes to blood pressure lowering effect of ACE-Is⁽²⁾.

Hypercholesterolemia is a major player for the development of coronary heart disease, which is leading cause of morbidity and mortality in many countries. It is important reversible risk factor for the development of acute thrombotic complications of atherosclerosis like myocardial infarction and ischemic stroke⁽³⁾. Platelets and coagulation factors are strictly involved in the genesis of such thrombotic events. Elevated plasma cholesterol level is significantly

associated with hypercoagulability, as well as an increase in the platelets activation arising from an increase in cholesterol/phospholipid ratio, thromboxane A₂ biosynthesis, platelets alpha- adrenergic receptors density, plasma membrane and cytosolic calcium level and through inhibition of Na⁺/H⁺ antiport. Therefore, hypercholesterolemia is associated with enhancement of thrombotic and a reduced fibrinolytic state⁽⁴⁾.

Recently, a crucial role in the regulation of hemostasis has been attributed to RAS system. In addition to their primary indications, hypertension and congestive heart failure, ACE-Is have pleiotropic effects which are under active investigations⁽⁵⁾. It has been demonstrated that captopril administered to patients after myocardial infarction, significantly decreased the frequency of recurrent thrombosis. Moreover other ACE-Is namely ramipril in survivors with clinical evidence of heart failure and trandolapril in patients with left ventricular dysfunction also reduced mortality after myocardial infarction^(6,7). These observations were confirmed by studies demonstrating the prothrombotic activity of angiotensin II, the main active peptide of RAS, Angiotensin II promotes thrombosis by increasing the production and secretion of plasminogen activator inhibitor type 1 (PAI-1) and augments the expression of tissue factor (TF)⁽⁸⁾. It has been suggested that the beneficial effect of ACE-Is on hemostasis depend on tissue accumulation of bradykinin⁽⁹⁾. Although ACE-Is have been broadly applied in hypertension, there are still few records of their effectiveness on hemostasis, especially in hyperlipidemia⁽¹⁰⁾. Therefore, the present study aims at investigating the effect of ACE-Is on hemostasis in hyperlipidemia.

Materials and Methods

The study was conducted on twenty-eight male Sprague-Dawley rats aged 17-19 weeks. Their weight was 175-170 g. Animals were housed in a room with a 12:12 hours light/dark cycle, in group cages as appropriate, and were got free access to tap water and libitum. After two weeks of acclimatization, the animals were randomized into four groups each containing seven rats: group1, 2,3 and 4. Group 1 was fed a standard chow diet and serve as normal diet control while group 2,3 and 4 received atherogenic diet (4% cholesterol-enriched diet)⁽¹¹⁾ for eight weeks. Animals in group 2,3 and 4 were treated with distilled water (lipidemic control), captopril and enalapril for the next four weeks respectively. Captopril was used in a dose of 2x25 mg/kg/day P.O. Enalapril was given in a dose of 15 mg /kg/day P.O.⁽¹²⁾.

At the end of the experiment, the animals were sacrificed and blood sample (2.5 ml) was obtained from the heart for determination of prothrombin time (PT), activated partial thromboplastin time (aPTT), plasma fibrinogen, platelets count and total serum cholesterol (TSC)

The blood sample was divided into three parts: A 1 ml of blood was placed in a tube containing sodium citrate as anticoagulant. The volume ratio of blood to sodium citrate is 9:1. Blood had centrifuged for 20 minutes at speed of 2500 rpm. The plasma was used to determine fibrinogen, PT and aPTT by routine laboratory assays. A 0.5 ml was placed in tube that contains EDTA as anticoagulant in 9:1 volume ratio. Blood film was prepared for counting platelets. The last 1 ml of blood was placed in serum tube and left to stand for 30 minutes. The serum was prepared via centrifugation at 3000 rpm for 10 minutes to determine TSC.

Statistical analysis

The data expressed as mean \pm SEM unless otherwise stated. Statistical analysis had been done by using paired t-test and ANOVA.

Significant difference was set at $\alpha=0.05$.

Results

Total serum cholesterol

In comparison with normal diet control, total serum cholesterol was increased more than three times in rats fed 4% cholesterol-enriched diet for eight weeks. This increase was statistically significant ($P<0.05$). Figure (1)

Animals that were treated with captopril and enalapril for 4 weeks did not show significant reduction ($P>0.05$) in total serum cholesterol compared to lipidemic control. Table(1)

Plasma fibrinogen

After 8 weeks of 4% cholesterol-enriched diet, the animals showed statistically significant rise ($P<0.05$) in plasma fibrinogen levels compared to normal diet control. The observed plasma fibrinogen levels in group1 and group 2 were (183.6 \pm 6.4, 438.72 \pm 9) mg/dl respectively. Figure (2).

Compared to lipidemic control, hyperlipidemic rats treated with captopril and enalapril for 4 weeks showed significant fall in plasma fibrinogen levels ($p<0.05$). Plasma fibrinogen levels were (438.72 \pm 9,

233.5 ±8.8 and 240 ±11.3) mg/dl in group 2, 3, and 4 respectively. Table(1)

Prothrombin time (PT)

Hyperlipidemic rats treated with captopril and enalapril for 4 weeks showed significant prolongation of PT (p<0.05) Table(1). The largest increase was observed in enalapril treated group (9.07 sec.) There were insignificant differences between treatment means of PT.

Activated partial thromboplastin time (aPTT)

In hyperlipidemic rats, four weeks of treatment with captopril and enalapril caused significant prolongation of aPTT (p<0.05) Table(1). Although the largest increase was seen in captopril treated group (15.62 Sec.), there were no significant differences between treatment means of aPTT.

Platelets counts

Eight weeks of treating hyperlipidemic rats with captopril and enalapril did not significantly alter the platelets counts (p>0.05). Table(1)

Figure (1) :Changes in total serum cholesterol level (mg/dl) in rats fed 4% cholesterol-enriched diet for 8 weeks versus normal diet control



Figure (2) :Changes in plasma fibrinogen level (mg/dl) in rats fed 4% cholesterol-enriched diet for 8 weeks versus normal diet control



Table(1):Effects of 4 weeks captopril and enalapril treatment on total serum cholesterol I(TSC), plasma fibrinogen, prothrombin time (PT) and activated partial thromboplastin time (aPTT) in hyperlipidemic rats .

Group	TSC mg/dl	Plasma fibrinogen mg/dl	PT Sec.	aPTT Sec.	Platelets count × 10 ⁹ /L
Lipidemic control	226.45 ± 18.3	438.72 ± 9	9.83 ± 0.2	21 ± 0.5	175.8 ± 1.5
Captopril 2×25 mg/kg/day	208.8 ± 16.9	*233.5 ± 8.8	*17.2 ± 0.5	*36.62 ± 0.9	182.12 ± 3.3
Enalapril 15 mg/kg/day	200.1 ± 24.2	*240 ± 11.3	*18.9 ± 1	*34.9 ± 1.4	179.5 ± 2.4

* = P<0.05

Discussion:

Beyond their blood pressure lowering potential, some antihypertensive agents may exhibit a number of non-hemodynamic effects such as changes in serum electrolytes, lipid and carbohydrate metabolism, endothelial function, vascular smooth muscle and cardiomyocytes growth and possible on coagulation / fibrinolysis balance⁽¹³⁾. Recently, a crucial role in the regulation of hemostasis has been attributed to RAS system. In addition to their primary indications, hypertension and congestive heart failure, ACE-Is have pleiotropic effects which are under active investigations⁽⁵⁾.

Hypercholesterolemia is important risk factor for the development and progression of atherosclerosis .It is not only linked to an increased risk of atherosclerosis but also to develop acute thrombotic complications of atherosclerosis like myocardial infarction and ischemic stroke⁽³⁾. Elevated plasma cholesterol level is associated with hypercoagulability and an increase in platelets activation arising from an increase in cholesterol/phospholipid ratio, thromboxane A2 biosynthesis, platelets alpha- adrenergic receptors density, plasma membrane and cytosolic calcium level and also through inhibition of Na⁺/H⁺ antiport⁽⁴⁾.

It has been observed that ACE-Is possess, alongside the hypotensive effect, also antithrombotic and fibrinolytic impact. ACE-Is decrease blood pressure by reducing vascular resistance. Therefore, it is not unlikely that captopril and enalapril affect primary hemostasis through their effect on blood vessels. There is growing number of clinical and

experimental data showing the beneficial role of ACE-Is in the regulation of hemostasis, yet they all refer to hypertension⁽⁷⁾. In the present study, we evaluated the effect of selected ACE-Is on hemostasis in hyperlipidemia.

The present study demonstrates insignificant reduction in total serum cholesterol levels ($p > 0.05$) in both captopril and enalapril treated groups. Laith AL-Husseini 2002⁽¹⁴⁾ found a similar result .

In the present study, we observed that the plasma fibrinogen level was significantly increased ($p < 0.05$) in hyperlipidemic rats. This finding is similar to a finding by Levenson et al, 1995⁽¹⁵⁾, Alessandrie et al, 1996⁽¹⁶⁾, Kristensen et al, 1998⁽¹⁷⁾ and Bassim Al Sheibani, 2003⁽¹⁸⁾, they found a significant increase in plasma fibrinogen level in hyperlipidemia. The proposed explanation is the prolonged hyperlipidemia may enhance the atherogenic process and thence rise in plasma fibrinogen level⁽¹⁹⁾.

In hyperlipidemic rats, this study shows that the platelets count were unaffected ($p > 0.05$) by captopril and enalapril treatment. Sechi et al, 2000⁽²⁰⁾ and Hyder Al Mousawi, 2005 found a similar result⁽²¹⁾.

Our current study showed that treatment of hyperlipidemic rats with captopril 2x25 mg/kg/day and enalapril 15 mg/kg/day resulted in a significant fall ($p < 0.05$) in plasma fibrinogen level. These findings agree with the findings of Moser et al, 1997, they had found that captopril caused a decrease in elevated plasma fibrinogen⁽²²⁾. Li-Saw-H et al, 2001 found that enalapril did not significantly affect plasma fibrinogen level⁽²³⁾.

This study demonstrates a significant prolongation of PT and aPTT ($p < 0.05$) in hyperlipidemic rats treated with captopril 2x25 mg/kg/day and enalapril 15 mg/kg/day. Our current findings are consistent with reports by Kucharewicz et al, 2002. They found that captopril prolong PT and aPTT⁽²⁴⁾. Buczko et al, 2004⁽¹²⁾ and Hyder Al Mousawi, 2005⁽²⁰⁾ showed that ACE-Is have no significant effect on PT and aPTT.

The postulated mechanism by which ACE-Is affect hemostasis is through prevention of angiotensin II synthesis. Angiotensin II, main active peptide of RAS, has prothrombotic activity. Angiotensin II promotes thrombosis by increasing the production and secretion of plasminogen activator inhibitor type 1 (PAI-1) and augments the expression of tissue factor (TF)⁽⁸⁾. Additionally, it has been suggested that the beneficial effect of ACE-Is on hemostasis depend on tissue accumulation of bradykinin. ACE-Is, by blocking kininase II, suppress bradykinin degradation and through activation of bradykinin receptors, increase nitric oxide (NO), prostacyclin (PGI₂) and tissue plasminogen activator (t-PA) release⁽⁹⁾. It is also suggested that inhibition of adhesion

and aggregation of platelet is essentially caused by NO and PGIs released under the influence of ACE-Is^(25,26).

We concluded that captopril and enalapril decrease the elevated plasma fibrinogen level in hyperlipidemia. They also prolong PT and aPTT; therefore captopril and enalapril have favorable effect on hemostasis. In addition, captopril and enalapril cause tendency of TSC to fall, though it is not statistically significant so they might share in decreasing the thrombogenic potential of atherosclerosis.

References

- 1-Griendling KK, Murphy TJ, and Alexander RW: Molecular biology of the renin-angiotensin system. *Circulation*, 1993,87,1816-1828.
- 2-Burnier M, Brunner HR: Angiotensin II receptor antagonist. *Lancet*, 2000; 355:637-654.
- 3-Pucceti L, Bruni F, Di-Renzo M, Bova G, Cercignani M, Indanza A, Auteri A, Pasqui AL: Hypercoagulable state in hypercholesterolemic subjects assessed by platelet-dependent thrombin generation: in vitro effect of cerivastatin. *Eur.Rev.Med. Pharmacol.Sci.* 1999 sep-oct; 3(5): 197-204.
- 4-Nofer JR, Tepel M, Kehrel B, Wierlille S, Walter M, Seedorf U, Zidek W, Assmann G; Low density lipoproteins inhibit the Na/H antiport in human platelets: a novel mechanism enhancing platelet activity in hypercholesterolemia. *Circulation* 1997; 95:1370-1377.
- 6-Pawlak R, Chabielska E, Golasowski J, Azzadin A, Buczek W; Nitric oxide and prostacyclin are involved in antithrombotic action of captopril in venous thrombosis in rats. *Thromb Haemost*, 1998; 79: 1208-1212.
- 7-The AIRE studies Investigators. Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. The acute infarction ramipril efficacy. *Lancet* 1993; 342:821-82.
- 8-Vaughan DE, Lazos SA, Tong K. Angiotensin II regulates the expression of plasminogen activator inhibitor-1 in cultured endothelial cells. A potential link between renin-angiotensin system and thrombosis. *J Clin Invest* 1999; 95:995-1001.
- 9-Gryglewski RJ. Interaction between endothelial mediators. *Pharmacol Toxicol* 1995;77:1-9.
- 10- Kober L, Torp-Pedersen C, Carlesn JE, Bagger H, Eliassen P, Lyngborg K, Videback J. A clinical trial of the angiotensin-converting-enzyme inhibitor trandolapril in patient with left ventricular dysfunction after myocardial infarction. Trandolapril Cardiac Evaluation (TRACE) Study Group. *N Engl J Med* 1995;333: 1670-1676.

- 11-Tobian L, Jahner TM, Johnson MA. High k⁺ diets markedly reduce atherosclerosis in apo E-deficient animal. *Circulation* 2001;Jan 23;103(3):448-54.
- 12- Buczko W, Kubik A, Kucharewicz I, Chabielska E. Antithrombotic effect of captopril and enalapril in young rats. *Pharmacol Reports* 2004;56:97-104.
- 13- Laith AL-Husseini .Effect of some antihypertensive drugs on serum lipid profile .MSc. Thesis 2002.
- 14-Lee AJ . The role of rheology and hemostatic factors in hypertension. *J Hum Hypertens* 1997 ;11:767-776.
- 15-Levenson J, Giral P, Razavian M, Garipey J, Simon A. Fibrinogen and silent atherosclerosis in subjects with cardiovascular risk factors. *Arterioscl Thromb Vasc Biol* 1995;15:1263-1269.
- 16-Alessandrie C, Basili S, Maurelli M, Bracaglia D, Andreozzi P, Pergolini M, Cordova C. Relationship between prothrombin activation fragment F1+2 and serum cholestrol. *Haemostasis* 0996 Jul-Aug; 26(4):214-9.
- 17-Krestensen B, Malm J, Nilsson T, Hultdin J, Carlberg B, Olsson T. Increased fibrinogen levels and acquired hypofibrinolysis in young adults with ischemic stroke. *Stroke* 1998; 29:2261-2267.
- 18- Bassim I Al Sheibani. A study of the effect of statins on some coagulation parameters in normolipidemic and hyperlipidemic rats. MSc. Thesis 2003.
- 19-Ijem J, Granlie C. More than cholestrol. The complexity of coronary artery disease. *S D J Med* 2000 Nov;53(11):489-91.
- 20-Sechi LA, Zingaro L, Casaccio D, and De Marchi. Relationship of fibrinogen levels and haemostatic abnormalities with target organ damage in hypertension. *Hypertension* Dec.1 2000;36(6):978-985.
- 21- Hyder I Al Mousawi.Effects of some antihypertensive drugs on coagulation parameters and serum lipid levels in hypertensive patients. MSc. Thesis 2005.
- 22-Moser L, Callan KS, Cheung AK, Stoddard GJ, Munger MA. ACE inhibitors effects on platelets function in stagesI-II hypertension. *J Cardiovasc Pharmacol* 1997;30:461-467.
- 23-Li-Saw-Hee FL, Beevers DG, Lip GY. Effects of antihypertensive therapy using enalapril or losartan on haemostatic markers in essential hypertension. A pilot prospective randomized double-blind parallel groups. *Int J Cardio* 2001 May;78(3):241-6.
- 24- Kucharewicz I, Pawlak R, Matys T, Pawlak D, Buczko W. Antithrombotic effect of captopril and losartan is mediated by angiotensin (1-7). *Hypertension* 2002; 40:774-779.

- 25- - Buczko W, Matys T, Kucharewicz I, Chabielska E. The role of endothelium in antithrombotic effect of the renin-angiotensin system blocked. *J Physiol Pharmacol* 1999;4:499-507.
- 26-Farthy R, Carretero OA, Ho KL, Scicli AG. Role of kinins and nitric oxide in the effect of angiotensin-converting enzyme inhibitors on neointima formation. *Circ Res* 1993; 87:1202-1210.