In Vivo Effect of Losartan on Platelet Aggregation in Patients with Hypertension

LOSARTANIN HİPERTANSİF HASTALARDA TROMBOSİT KÜMELENMESİ ÜZERİNE ETKİSİ

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Abstract

Purpose: Losartan has been found to inhibit platelet aggregability to some extend in in-vitro experiments. There were conflicting results about the in vivo effects of Angiotensin II receptor blocker losartan. We sought to clarify the in vivo effect of losartan on platelet aggregation.

Material and Methods: 40 grade I essential hypertensive patients were treated with losartan for three weeks. Platelet aggregation tests with adenosine diphosphate (ADP) and ristocetin were analyzed and compared before and at the end of the study.

Results: Losartan effectively decreased systolic and diastolic blood pressure (BP). Mean SBP before and after treatment were 159,6±12,8 mmHg and 149,2±17,3 mmHg respectively. Mean DBP decreased from 93,7±8,2 mmHg to 87,7±10,3 mmHg after treatment. Results of platelet aggregation tests with ADP and ristocetin were not significantly different when both rate and amplitude of maximal aggregation was concerned. Peak platelet aggregation with ADP regarding lowest light transmission in the aggragometer were 59,8±24,3% before and 58,3±18,1% after the treatment. The same variables with ristocetin were 66,8±21,6% and 60,8±23,3% respectively.

Conclusions: In vivo effects of losartan on platelet aggregation with ADP and ristocetin were insignificant.

Key Words: Losartan, platelet aggregation, hypertension

Özet

Amaç: Losartan ile yapılan in vitro çalışmalarda trombosit kümelenmesinin azaldığı gösterilmiştir. İn vivo çalışmaların sonuçları ise zıt sonuçlar içermektedir. Bu çalışmamızda losartanın trombosit kümelenmesi üzerine in vivo etkisini araştırmayı hedefledik.

Gereç ve Yöntemler: Evre I esansiyel hipertansiyonlu 40 hasta losartan ile 3 hafta tedavi edildi. Adenozin Difosfat (ADP) ve ristosetin ile tedavi öncesi ve sonrası yapılan trombosit kümelenme testleri karşılaştırıldı.

Bulgular: Losartan ortalama sistolik (SKB) ve diyastolik (DKB) kan basıncını tedavi sonunda anlamlı olarak düşürmüştür (p<0,001). Ortalama SKB ve DKB değerleri tedavi öncesi ve sonrası sırasıyla 159±12,8, 149±17,3 ve 93,7±8,2 , 87,7±10,3 bulundu. Tedavi öncesi ve sonrası, ADP ve ristosetin ile yapılan trombosit kümelenme testleri arasında istatistiksel olarak farklılık saptanmadı. Agragometre ile en az ışık geçirgenliğinin saptandığı noktanın en yüksek kümelenme yüzdesi olarak alındığı ADP ile yapılan test sonucunda ortalama değerler tedavi öncesi ve sonrası 59,8±24,3 ve 58,3±18,1 olarak bulundu. Aynı değişkenler ristosetin ile 66,8±21 ve 60,8±23,3 olarak saptandı.

Sonuçlar: Losartanın ADP ve ristosetin ile yapılan test sonuçlarına göre trombosit kümelenme işlevi üzerine azaltıcı etkisi istatistiksel olarak anlamlı değildir.

Anahtar Kelimeler: Losartan, trombosit kümelenmesi, hipertansiyon

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Losartan has been found to prevent platelet aggregation in hypertensive rats via blocking the thrombaxane A2/prostaglandin H2 (TXA2/PGH2) receptors.[1] The Evaluation of Losartan In The Elderly (ELITE) study demonstrated that losartan decrease sudden death[2] in which thrombus formation through platelet adhesion and aggregation plays an important role.[3] Different studies demonstrated a strong correlation between hypertension and coagulation disorders.[4-5] The two most encountered complications of hypertension; myocardial infarction and stroke are

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pathogenically related to thrombus formation[6]. Hypertensive patients are subject to endothelial dysfunction and increased platelet aggregability[7]. The present study is designed to assess the efficacy of losartan on the platelet aggregation in hypertensive patients.

Material and Methods

Patients: This study was performed in 40 subjects; 27 women and 13 men; mean age were [mean ± SD] 52,3 ± 9 ranging from 37 to 68 years, with stage I essential hypertension. Blood pressure measurements were obtained after subjects rested in the supine position for a minimum of 15 minutes in a quiet examining room maintained at an ambient temperature, with the same sphyingomanometer. Three measurements were done with two minute intervals and mean value was calculated. Exclusion criteria were; subjects with coronary artery disease, congestive heart failure, known secondary causes for hypertension; an acute illness of any type; previously documented thrombotic events (e.g., deep vein thrombosis, myocardial infarction, or stroke), or defined hypercoagulable state; major surgery in the past 3 months; current or past exposure to antiplatelet therapy, nonsteroidal anti-inflammatory drugs, lipid-lowering therapy, antidepressants, or oral contraceptives, current smokers, subjects with diabetes mellitus, chronic renal insufficiency (serum creatinine >150μmol/l), cancer patients, subjects with abnormal biochemical laboratory findings(such as serum potassium <3.5 or >5.5 mmol/1, transaminases over two times normal values), subjects with known allergy to losartan potassium, and subjects with worsening of kidney function while receiving angiotensin-converting enzyme inhibitor treatment. Obese patients having arm circumfarence over 41 cm were also excluded from the study.

Study Design: All subjects underwent a screening physical examination, urinalysis, and laboratory tests (blood counts and serum biochemistry, including renal function and electrolytes) which were all normal. After a wash-out period of all drugs for ten days, platelet aggregation tests with ADP and ristocetin, 12 lead electrocardiogram, chest X-ray and biochemical analyses were performed. Initial blood pressures were measured and losartan 50 mg/daily started. Patients were asked to take their medication at 10 o'clock in the morning. Platelet aggregation tests and blood pressure measurements were repeated after three weeks of therapy.

Laboratory studies: Venous blood withdrawn (9 ml) and anticoagulated with 1 ml of 3,8% trisodium citrate. Platelet-rich plasma (PRP) prepared by centrifugation of the blood at 1500 rpm for 15 minutes at 20°C, as described elsewhere.[8] The supernatant were drawn to another tube, and remaining PRP again centrifuged at 2500 rpm for 5 minutes to get platelet-poor plasma (PPP).

After waiting 30 minutes at 24°C, light permeability was adjusted to 100% by using PPP of the same patient. 450μL of PRP incubated at 37°C for 2 minutes and stirred again for 2 minutes in the aggregometer (Chrono-log 500 CA, USA). Changes in optical density were recorded for 6 minutes after platelets were stimulated with 50μL ADP, and ristocetin (Sigma diagnostics; USA) at concentrations of 2×10^{-4} mol/L and 1×10^{-4} mol/L respectively.

A turbidimetry curve, expressing the relation between the amplitude and rate of the aggregation was calculated for each subject's sample (Figure-1). The lowest light transmission at the end of the sixth minute was recorded as the maximal aggregation percent (%). The time that 50% of maximal aggregation occured was recorded as aggregation rate (sec).

Ethics

The protocol of the study was approved by the Ethical Committee of Scientific Research Programmes of Istanbul University Cerrahpasa Medical School. Each subject had signed a consent form.

Statistics

Statistical package for social sciences (SPSS ver. 10,0) software was used for analysis. Results were expressed as means ±SD. Quantitative data

Figure 1. The turbidimetry curves of three patients with ADP and ristocetin.

were analysed using Student's paired t-test and a value of P<0,05 was considered statistically significant.

Results

Mean blood pressure before the study was 159,62 ± 12,87 mmHg for systolic and 93,75 ± 8,22 mmHg for diastolic BP. The measurements after three weeks of treatment resulted a mean SBP of 149,25± 17,34 mmHg and a mean DBP of 87,75 ±10,37 mmHg which was significant. Losartan tratment did not interfere the platelet aggregation extent and rate significantly after plasmas stimulated with ADP and ristocetin (Table 1). Peak platelet aggregation with ADP regarding lowest light transmission in the aggragometer was 59,8±24,3% before and 58,3±18,1% after the treatment. The same variables with ristocetin were 66,8±21,6% and 60,8±23,3% respectively. The mean aggregation rate with ADP was 89,5±37,6 sec. before and 94,5±26,3 sec. after the treatment. The same variables with ristocetin were 102,3±38,3 sec. and 96,5±39,3 sec. respectively. (Figures 2 and 3).

Discussion

There are two subgroups of angiotensin (AT) II receptors. Stimulation of AT1 receptors cause vasoconstriction, increase in myocardial contractility, aldesteron secretion, incresase in glomeruler filtration rate, renal blood flow and increase in myocardial and vascular hypertrophy where as stimulation of AT 2 receptors has antiproliferative, antihypertensive and cardioprotective effects. The pharmacological blockade of the renin-angiotensin system (RAS) by ACE-inhibitors and by AT1-receptor antagonist represents an established, beneficial, and successful treatment of patients with arterial hypertension, chronic renal failure, and atherosclerosis. After the development of ACE inhibitors, benzyl-substituted imidazoles were developed in search for a more specific blockade of the RAS by blocking Ang II effects more selectively at the receptor level[9] Losartan is the first non-peptide AT1 receptor antagonist that has been used successfully in hypertensive patients. Losartan is a pro-drug and the main hepatic metabolite is EXP3174, which is 10-15 times more potent as an AT1 receptor antagonist than losartan itself.[10]

Clinical data suggest that essential hypertension is associated with platelet hyperaggregability.[11] Antiplatelet effects of antihypertensive drugs is a topic of intense investigation. Isradipin, amlodipin and high dose diltiazem can inhibit platelet aggregation in a significant extend.[12-14] The results

Table 1. Effects of losartan treatment on hemodynamic variables and platelet aggregability

		Before treatment	After treatment	P value
Systolic BP (mmHg)	40	159,6±12,8	149,2±17,3	<0,001
Diastolic BP (mmHg)	40	93,7±8,2	87,7±10,3	<0,001
ADP-amplitude (%)	40	59,8±24,3	58,3±18,1	0,362
ADP-rate (sec)	40	89,5±37,6	94,5±26,3	0,463
Ristocetin amplitude (%)	40	66,8±21,6	60,8±23,3	0,381
Ristocetin-rate (sec)	40	102,3±38,3	96,5±39,3	0,448

Values are expressed as mean±SD of data obtained in 40 essential hypertensive subjects before and after three weeks of therapy.

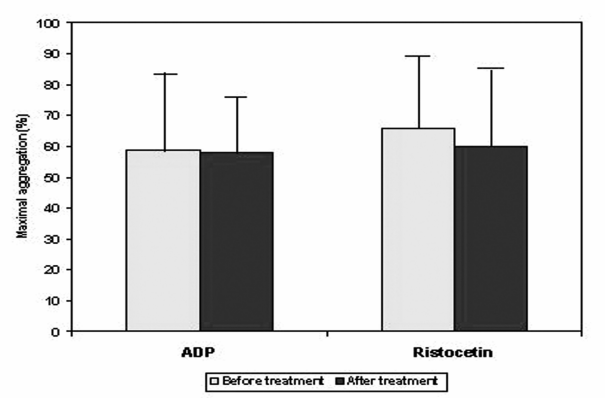

Figure 2. Effects of losartan treatment on platelet aggregation before and after three weeks of therapy, assessed as the percentage of light loss 6 min after plasmas were stimulated with ADP and ristocetin. Data are means±SD of 40 subjects.

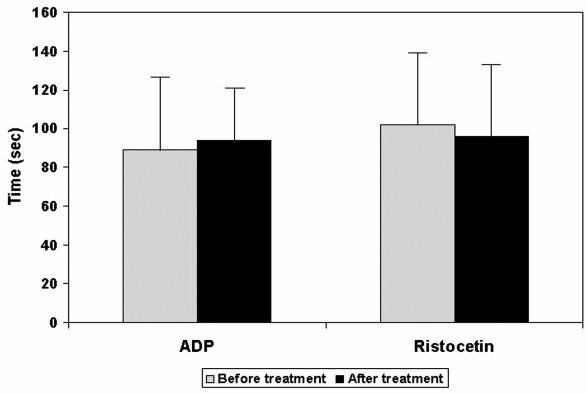

Figure 3. Effects of losartan treatment on platelet aggregation before and after three weeks of therapy, assessed as the time needed to reach 50% of maximal aggregation after plasmas were stimulated with ADP and ristocetin. Data are means±SD of 40 subjects.

with ACE inhibitors were conflicting. Swartz et al demonstrated that captopril can decrease platelet aggregability where as Moser et al found no significant antiaggregatory effect with captopril, enalapril and fosinopril.[15,16] Keidar et al demonstrated a 31% decrease in platelet aggregation after fosinopril treatment where as Hale et al. showed a similar effect in primates only when fosinopril was administered with pravastatin[17,8]. Gupta et al. could not found any significant effect on platelet aggregation with quinapril.[18]

Findings in the literature suggest that inhibition of platelet aggregation may be another factor differentiating the mode of action of angiotensin-converting enzyme inhibitors from Ang II antagonists. Li et al showed that losartan and its active metabolite EXP 3174 competed with the TXA2/prostaglandin endoperoxide H2 receptor in isolated canine coronary arteries, in the circulation of the rat with spontaneous hypertension, and in human platelet-rich plasma in vitro.[19,1] Losartan can block pulmonary hypertension induced by TXA2 infusion.[20] This is a direct action independent of agents relased by the endothelium such as nitric oxide.[19] Losartan significantly reduces platelet aggregation induced by the TXA2 analogue U46619[21]. Losartan diminished collagen–induced human platelet aggregation to a similar extent as aspirin, while EXP3174 and valsartan showed much weaker action.[22] The AT1-receptor antagonist losartan elicits antiinflammatory properties via its EXP3179 metabolite by blocking COX-2 mRNA upregulation and COX-2–dependent TXA2 and PGF2 generation in vitro. Moreover, EXP3179 abolishes dose-dependently arachidonic acid (AA)–induced platelet aggregation, suggesting an inhibitory effect at the COX enzyme. Losartan elicit antiaggregatory effects independent of blockade at the AT1 receptor in clinically relevant doses.[23]

The competitive inhibitory effects of losartan on the TxA2 receptor are not a class effect because neither candesartan[19] nor valsartan[24] displayed these effects. On the other hand, irbesartan may share the effects of losartan on inhibiting the TxA2 receptor.[24] Nunez et al demonstrated that U46619-stimulated platelet activation was significantly reduced by losartan in a dose-dependent manner. Only maximal doses of valsartan ($5x10^{-6}$ mol/L), reduced U46619-induced platelet activation. The active form of candesartan cilexetil and candesartan (CV-11974), failed to modify platelet activation. Losartan reduced the binding of [(3)H]-U46619 to platelets, an effect that was observed to a lesser extent with valsartan but not with CV-11974.[25]

Although antithrombotic effect of losartan occurs at doses 1,000-fold higher than those required for binding to vascular AT1 receptors[26] Pavel et al. demonstrated directly that losartan induced a statistically significant reduction in the extent of thrombin-mediated platelet aggregation within 4 weeks after initiation of therapy at a dose of 50 mg.[27] Losartan is superior over EXP 3174 and valsartan in inhibiting thrombocyte function and platelet-dependent thrombosis possibly due to a stronger action on the thromboxane A2/prostaglandin H2 receptor.[28]

The present study evaluates the in vivo effect of losartan on platelet aggregability. Pavel et al found that losartan significantly reduces platelet aggregation where as Owens et al could not confirm this finding.[27,29] Pavel et al. used SFLRRN-NH_2 trombin receptor-activating peptide where as Owens at al. used ADP and thromboxaneA_2 analog U46619. Both studies were performed in small patient groups; 10 and 24 patients respectively. The difference may aroused by the different reagents used. Owens et al compared test results with placebo class. In our study, the effect of losartan treatment was insignificant in reduction of platelet aggregation when platelets were stimulated with ADP and ristocetin. Further larger scale studies should be performed in order to evaluate in vivo effects of losartan on platelet aggregation.

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