



Inhibition of platelet aggregability by losartan in essential hypertension

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Epidemiologic evidence documents that there is a link between hypertension and coagulation disorders^{1,2}. Among the latter are increased platelet aggregability³ and vascular endothelial dysfunction⁴. Evidence suggests a contribution of the renin-angiotensin system to the pathogenesis of atherosclerosis⁵ and to the development of a prothrombotic stage in hypertensive subjects⁶. Angiotensin II (Ang II) increases production and secretion of plasminogen activator inhibitor type 1 (PAI-1) from vascular endothelial cells⁷, whereas losartan inhibits the vasoconstrictor and platelet aggregation effects induced by thromboxane A₂ (TxA₂)⁸⁻¹⁰. The possibility that chronic therapy with an Ang II antagonist may decrease platelet aggregability, thus contributing to amelioration of the prothrombotic stage associated with hypertension has not been studied in human subjects. Therefore, we assessed the effects of 4-week losartan therapy on thrombin-mediated platelet aggregation and plasma markers of coagulation and fibrinolysis in subjects with stages I and II hypertension.

METHODS

Patients

This study was performed in 10 subjects (4 women and 6 men, mean age [mean \pm SD] 53 \pm 9 years, range 43 to 69) with stages I to II essential hypertension. Nine other healthy normotensive (\leq 140/90 mmHg) volunteers (39 \pm 11 years, range 24 to 55) participated as a time-control group. All subjects were recruited from the Hypertension and Vascular Disease Clinic in compliance with guidelines approved by the Wake Forest University Institutional Review Board/Human Subject Review Committee and after each subject had signed a consent form. Blood pressure measurements were obtained and venous blood withdrawn (10 ml) after subjects rested

in the supine position for a minimum of 15 minutes in a quiet examining room maintained at an ambient temperature between 25° C and 28° C. Four of the 9 subjects had newly diagnosed hypertension and had not received prior antihypertensive therapy. Exclusion criteria were: subjects with 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 \geq 1.8 mg/dl), known allergy to losartan potassium, and subjects with worsening of kidney function while receiving angiotensin-converting enzyme inhibitor therapy were also excluded from the study.

Study design

The effects of 4 weeks of losartan therapy on arterial pressure, thrombin receptor agonist-induced platelet aggregation, plasma levels of the von Willebrand factor (vWF) antigen, and PAI-1 antigen concentrations were determined in a prospective, double-blind (patients and hematology laboratory), placebo-controlled study. All subjects underwent a *screening* physical examination, urinalysis, and laboratory tests (blood counts and serum biochemistry, including renal function and electrolytes). During the study, participants were asked to abstain from taking aspirin or aspirin-containing medications and antidepressants, and were instructed to report any acute illness. Weekly visits were scheduled to assess changes in blood pressure, compliance with medications (pill counting), and adherence to avoidance of using over-the-

counter medications. After a 4-week washout period, all subjects entered a 2-week placebo run-in period, followed by a 30-day period of losartan therapy (50 mg once daily). Venous blood was obtained at the end of the washout period, both at weeks -2 and 0 of the placebo run-in period, and at week 4 of losartan therapy. In healthy time-control volunteers, blood sampling was obtained at the beginning of the study and again 30 days after.

Laboratory studies

Platelet responsiveness to the thrombin receptor-activating peptide SFLRRN-NH₂ (PMIS387, Bachem, California) was determined from platelet-rich plasma prepared by centrifugation of the blood at 900 rpm for 15 minutes at 20° C, as described elsewhere¹¹. Platelet aggregations were performed from platelet-rich plasma incubated at 37° C for 2 minutes and stirred for 60 seconds (1,100 rpm) in an aggregometer (PAP 4-C, Biodata, Horsham, Pennsylvania). Changes in optical density were recorded for 4 minutes after platelets were stimulated with the thrombin receptor agonist at concentrations ranging from 5 to 1,000 µmol/L. A 4-parameter logistic dose-response curve, expressing the relation between percent aggregation, rate, and final concentration of the thrombin receptor agonist was calculated for each subject's sample. Both maximal extent of aggregation and rate were determined for each concentration. The fitted curve allowed determination of the agonist concentration required to produce half-maximal response (EC₅₀)¹¹. Plasma concentrations of the PAI-1 and von Willebrand protein were determined using the Asserachrome enzyme-linked immunosorbent assay kits from Diagnostica Stage (Parsippany, New Jersey).

Statistical analysis

The primary end point of the study was the measurement of changes in thrombin receptor agonist-induced platelet aggregation between losartan and placebo periods. Descriptive analyses were performed by the calculation of means ± SD for continuous variables and proportions for categorical variables using the Student's *t* test and chi-square or Fisher's exact test. Multicollinearity between primary end points and potential confounders was excluded by correlation analysis. The primary end point was evaluated by analysis of variance with effects for period and treatment groups. Analysis was performed using the SAS statistical package (Cary, North Carolina).

RESULTS

Nine of the 10 patients initially enrolled in the study completed the trial without either adverse reactions or side effects. One subject was excluded from the study because of failure to discontinue antidepressant therapy. Treatment with losartan produced an 11.2% decrease in systolic blood pressure that was statistically lower (p = 0.01) than the values recorded during both the preceding placebo and washout periods (table 1). Changes in diastolic blood pressure were not significantly different between the end of the placebo and treatment periods (p > 0.05). The EC₅₀ for extent and rate of platelet aggregation induced by the thrombin receptor agonist averaged 0.95 ± 0.23 µmol/L and 1.04 ± 0.24% (mean ± SD), respectively, in 9 healthy volunteers at the initiation of the study. These values were not different from those recorded in study patients at weeks -2 and 0 of the placebo period (table 1).

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

	Washout Period (wk -6)	Placebo Period		Losartan therapy (wk 4)	p Value*
		Wk -2	Wk 0		
Systolic blood pressure (mmHg)	147 ± 15	149 ± 15	152 ± 18	135 ± 9	0.01
Diastolic blood pressure (mmHg)	87 ± 7	89 ± 7	90 ± 6	87 ± 10	0.23
Heart rate (beats/min)	72 ± 10	75 ± 11	73 ± 8	75 ± 8	0.48
TRA EC ₅₀ extent (µmol/L)	ND	0.95 ± 0.33	0.95 ± 0.34	1.14 ± 0.29	0.05
TRA EC ₅₀ rate (%)	ND	1.05 ± 0.28	1.03 ± 0.31	1.14 ± 0.28	0.05

*The values denote statistical differences at week 4 of losartan treatment compared with weeks -2 and 0 of placebo therapy. Values are expressed as mean ± SD of data obtained in 9 essential hypertensive subjects of corresponding time periods. ND: not determined; TRA: thrombin receptor agonist.

In contrast, table 1 also shows that losartan produced a significant increase in the EC₅₀ of thrombin receptor agonist-induced platelet aggregation extent and rate in hypertensive subjects. The reduction in platelet aggregability and the prolongation of the time required for platelet aggregation were significantly greater than values determined for either the same subjects at the end of the placebo run-in period or normotensive time-control subjects (fig. 1). Three of 9 patients had an increase in the EC₅₀ of thrombin receptor agonist-induced aggregation extent and rate during the placebo pretreatment period. Seven of 9 patients (78%) had a significant increase in thrombin receptor agonist-induced aggregation extent and rate 4 weeks after initiation of losartan therapy (fig. 1).

In healthy volunteers, plasma concentrations of PAI-1 (47.26 ± 26.30 ng/ml) and vWF (92.61 ± 71.83%) were within the range reported in other studies¹² and not different when measurements were repeated 30 days after (p < 0.05). Baseline plasma levels of PAI-1 in hypertensive subjects averaged 78.68 ± 50.40 ng/ml during the placebo period (fig. 2). Although these values were 66% higher than those found in healthy volunteers, the difference did not attain statistical significance (p = 0.12). Losartan had no effect on plasma levels of PAI-1 (fig. 2) and vWF (fig. 3) in hypertensive subjects. Correlation analysis of observed changes in thrombin receptor agonist EC₅₀ extent, age, gender, and changes in arterial pressure, PAI-1, and vWF were not statistically significant (table 2).

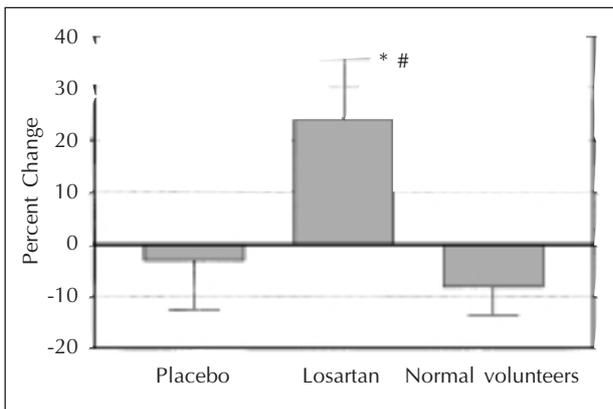


Fig. 1.—Increase in the EC₅₀ extent of platelet aggregation mediated by a selective thrombin receptor agonist in 9 hypertensive subjects given 50 mg/day of losartan over 4 weeks. The EC₅₀ for thrombin-induced platelet aggregation did not change in the same subjects over the 2-week placebo period. Thrombin-induced platelet aggregation remained unchanged in 9 normal volunteers during a 30-day time control period. Values are expressed as mean ± 1 SE. *,#p < 0.05 compared with placebo and normal volunteers.

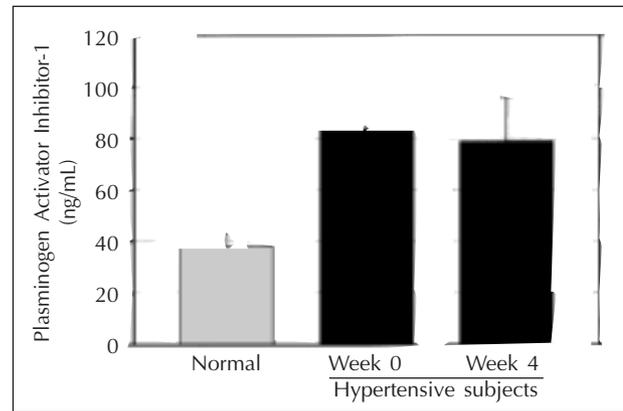


Fig. 2.—Differences in the plasma concentrations of plasminogen activator inhibitor-1 in 9 untreated healthy volunteers (stippled bar) and 9 hypertensive subjects at week 0 (solid bar, end of placebo period) and at week 4 of losartan treatment (solid bar, week 4). Values are expressed as mean ± 1 SEM.

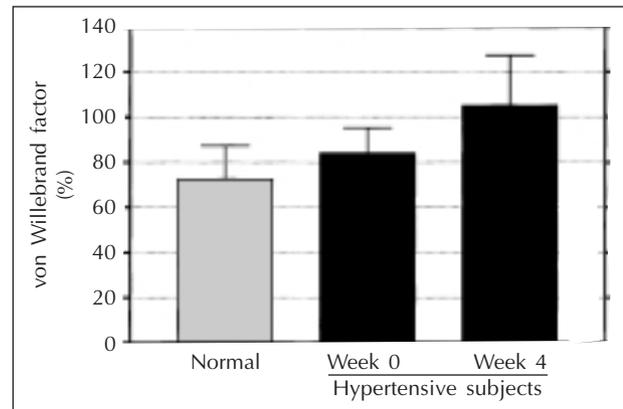


Fig. 3.—Differences in the plasma concentrations of von Willebrand factor in normal untreated healthy volunteers (stippled bar) and hypertensive subjects at week 0 (solid bar, end of placebo period) and at week 4 of losartan treatment (solid bar, week 4). Values are expressed as mean ± 1 SEM.

DISCUSSION

Treatment of hypertensive subjects over 4 weeks with losartan increased the EC₅₀ for thrombin-induced platelet aggregation by 25%. This novel effect of an Ang II antagonist after short-term standard-dose therapy in patients with mild to moderate essential hypertension was inferred from previous experimental studies¹³. Its significance was not fully appreciated because high doses of losartan were used to demonstrate this event⁸⁻¹⁰. This study now shows an antithrombotic effect of losartan in hypertensive subjects, which could be detected at initial treatment

Table II. Correlation analysis between changes in thrombin receptor agonist and laboratory variables

Variable	SBP	DBP	Age	Gender	PAI-1	vWF
Thrombin receptor agonist						
% EC ₅₀ extent						
R coefficient	-0.44	-0.21	0.39	0.17	0.09	0.20
p Values	0.24	0.59	0.11	0.49	0.82	0.61
Thrombin receptor agonist						
EC ₅₀ rate						
R coefficient	-0.33	0.03	0.28	0.29	0.13	0.39
p Values	0.39	0.94	0.26	0.24	0.74	0.29

Regression analysis (R value) of data obtained in 9 essential hypertensive subjects. DBP: diastolic blood pressure; SBP: systolic blood pressure.

doses and at the time at which the maximal antihypertensive effect of the drug was not fully achieved. The reduction in platelet aggregability was independent of changes in blood pressure and gender and not accompanied by modifications in the plasma levels of PAI-1 and vWF.

Clinical and laboratory data suggest that essential hypertension is associated with platelet hyper-aggregability¹⁴⁻¹⁶, reduced fibrinolytic activity¹⁷, and increased plasma concentrations of vWF¹⁸. In keeping with these findings, baseline levels of PAI-1 were 66% higher in hypertensive subjects than those found in normotensive subjects. The absence of a significant difference in baseline concentrations of PAI-1 and vWF between healthy volunteers and hypertensive subjects may be related to the small size of the sample and the inclusion of subjects with newly diagnosed hypertension and no associated clinical cardiovascular disease. Platelets play a major role in the induction of a «pro-thrombotic state» associated with high blood pressure⁶. Thrombin and TxA₂ are potent endogenous agonists of platelet aggregability, in part because thrombin stimulates the release of TxA₂ through activation of calcium ion-sensitive phospholipase A₂. In addition, Ang II stimulates the release of TxA₂ from vascular tissues via amplification of a G-protein-linked arachidonic acid pathway¹⁵. Li et al⁸ 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 hypertension⁹, and in human platelet-rich plasma *in vitro*¹³. The competitive inhibitory effects of losartan on the TxA₂ receptor are not a class effect because neither candesartan⁸ nor valsartan¹⁰ displayed these effects. On the other hand, irbesartan may share the effects of losartan on inhibiting the TxA₂ receptor¹⁰.

The concept that losartan may have a beneficial antithrombotic effect, independent of its antihypertensive property, has not been widely accepted

because it occurs at doses 1,000-fold higher than those required for binding to vascular AT₁ receptors^{8,9,13}. Although the argument has been posed that chronic therapy with losartan (80 to 120 mg oral dose) may expose platelets to circulating drug concentrations amounting to 1 µmol/L^{8,13} this study now shows 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. These findings suggest that inhibition of platelet aggregability may be a component of the therapeutic response associated with current dosing of the Ang II antagonist.

We took the precaution of determining the dose-response curve of the selective thrombin receptor agonist through a large range of concentrations to ensure an accurate quantification of the EC₅₀ dose. In this study, losartan elicited a 25% increase in the dose of thrombin receptor agonist required to produce a 50% response in the extent of aggregation compared with the values obtained in the same hypertensive subjects while taking placebo. The inclusion of similar measurements of platelet aggregability in a group of normal volunteers, spaced 30 days apart, was an added precaution to exclude random variations in the measurements related to time. It is intriguing that the significant increase in the EC₅₀ of aggregation could be demonstrated at the initial therapeutic dose of losartan and at a time at which the reduction in systolic but not diastolic blood pressure had attained statistical significance. Previous studies have emphasized that > 4 weeks are required to reach the nadir of the antihypertensive response^{19,20}. Analysis of variance incorporating potential confounders such as gender, blood pressure, and age excluded that the losartan-induced reduction in platelet aggregability was influenced by these variables.

Because it was not possible to obtain large quantities of platelet-rich plasma, we only evaluated the effects of losartan on thrombin receptor-mediated

aggregability. Therefore, the specific mechanism mediating the antithrombotic effects of the Ang II antagonist remains to be determined. Thrombin may trigger platelet aggregability by a direct action on platelets²¹ or by increased release of TxA₂^{7,22}. Losartan may have prevented binding of Ang II to platelets, thus preventing thrombin release. Alternatively, the antiaggregation effects of losartan may be related to blockade of TxA₂ receptors^{8,9}.

Antiplatelet effects of antihypertensive drugs are a topic of intense investigation. Diltiazem at high doses, 23 amlodipine²⁴, and isradipine²² have been reported to inhibit platelet aggregation. Conflicting results are reported for the effects of angiotensin-converting enzyme inhibitors on platelet function. Whereas captopril therapy may reduce platelet aggregation in response to adenosine phosphate, epinephrine, and collagen³, a prospective, randomized, double-blind, crossover study did not confirm these findings in hypertensive subjects treated with captopril, enalapril, or fosinopril¹⁵. Keidar et al²⁵ reported a 31% reduction of adenosine phosphate-induced platelet aggregation after fosinopril therapy, whereas Gupta et al²⁶ showed no effects of quinapril in essential hypertensive subjects. In contrast, a significant increase in the EC₅₀ of thrombin-mediated platelet aggregability was demonstrated in nonhuman primates only when given combined treatment with fosinopril and pravastatin¹¹. These findings suggest that inhibition of platelet aggregation may be another factor differentiating the mode of action of angiotensin-converting enzyme inhibitors from Ang II antagonists.

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