Review of the Molecular Pharmacology of Losartan and Its Possible Relevance to Stroke Prevention in Patients with Hypertension

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ABSTRACT

Background: The Losartan Intervention For Endpoint reduction in hypertension (LIFE) study found that a losartan-based regimen, compared with an atenolol-based regimen, resulted in a significantly lower risk of stroke in hypertensive patients with left ventricular hypertrophy, despite similar reductions in blood pressure.

Objective: The purpose of this review was to examine the molecular and pharmacologic mechanisms that may be associated with the different outcomes observed in the LIFE study.

Methods: A PubMed/MEDLINE search of English-language articles (1990 to February 2006) with the terms *angiotensin II antagonists* or *AIIAs* or *angiotensin receptor blockers* or *losartan* or *atenolol* or *beta blocker* and terms including, but not limited to, *atherosclerosis*, *left ventricular hypertrophy*, *carotid artery hypertrophy*, *fatty streaks*, *atrial fibrillation*, *arrhythmias*, *endothelial function*, *myocyte hypertrophy*, *myocardial fibrosis*, *platelet aggregation*, *tissue factor*, *plasminogen activator inhibitor-1*, *PAI-1*, *anti-inflammatory*, *uric acid*, or *oxidative stress*.

Results: Losartan's significant effect on stroke may be related to several possible mechanisms that are independent of blood-pressure reductions. These include improvements in endothelial function and vascular structure; decreases in vascular oxidative stress; reductions in left ventricular hypertrophy, reductions in myocardial fibrosis, or both; and modulation of atherosclerotic disease progression. Although some of these effects may be shared by other angiotensin II receptor antagonists (AIIAs), and perhaps other antihypertensive classes (eg, angiotensin-converting enzyme inhibitors), the ability of losartan to lower serum uric acid levels—a proposed independent risk factor for cardiovascular disease—appears to be a molecule-specific effect. Alternative explanations of the results of the LIFE study have also been hypothesized, including inappropriate choice of atenolol as an active comparator and differences in central pulse pressures between study groups.

Conclusions: This review of the literature suggests that losartan (and perhaps other AIIAs) may possess a number of properties, independent of its antihypertensive effects, that may be associated with decreased vulnerability of the plaque, myocardium, and blood. (*Clin Ther.* 2006;28:832–848) Copyright © 2006 Excerpta Medica, Inc.

Key words: stroke, angiotensin II antagonists, losartan, atenolol, β-blockers, angiotensin II receptor, atherosclerotic plaque, hyperuricemia, arrhythmias, thrombosis, inflammation.

INTRODUCTION

Despite evidence of the importance of reducing blood pressure (BP), hypertension continues to be one of the most commonly occurring diseases in humans.[1] Stroke is the most frequent complication in hypertensive patients.[2] Indeed, hypertension has been considered an underlying factor in ~70% of strokes.[3] In 8 recently completed hypertension trials including 55,110 patients, 2057 strokes and 1603 myocardial infarctions (MIs) were reported.[3] The relationship of stroke mortality to BP is strong and direct, regardless of age.[4]

In the Losartan Intervention For Endpoint reduction in hypertension (LIFE) study in hypertensive patients with left ventricular hypertrophy (LVH), the group of patients who received losartan had a 13.0% relative risk reduction in the occurrence of the primary composite end point of cardiovascular (CV) mortality,

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stroke (fatal and nonfatal), and MI (P = 0.021) and a 24.9% relative risk reduction in fatal or nonfatal stroke (P = 0.001) compared with patients receiving an atenolol-based regimen.[5] The LIFE study compared losartan-based therapy with atenolol-based therapy according to a double-blind, randomized, parallel-group design in 9193 patients aged 55 to 80 years with hypertension (sitting BP, 160–200/95–115 mm Hg) and LVH ascertained by electrocardiography.[5] Patients were initially randomized to losartan 50 mg plus placebo or atenolol 50 mg plus placebo. After 2 months, hydrochlorothiazide (HCTZ) 12.5 mg was added to the treatment regimen if the BP was not at or below goal BP (ie, <140/90 mm Hg). If, at month 4, the pressure was not adequately controlled, the dose of double-blind therapy was doubled to losartan 100 mg or atenolol 100 mg plus 12.5 mg HCTZ. In patients whose BP was still not controlled at month 6, additional open-label antihypertensive medication, including upward titration of HCTZ, was added to the treatment regimen. The dosage of HCTZ could be increased; additionally, or alternatively, any other antihypertensive drug (except angiotensin-converting enzyme inhibitors, angiotensin II type 1 receptor antagonists [AIIAs], or β-blockers) could be added at the discretion of the investigator. Outcomes favoring losartan were observed despite similar reductions in BP, suggesting that losartan's antistroke effects may have been independent of BP reduction. At the end of follow-up or last visit before a primary end point event, the mean sitting trough BP fell by 30.2/16.6 mm Hg in the losartan group and by 29.1/16.8 mm Hg in the atenolol group (P = 0.015 for systolic and P = NS for diastolic). Overall, the time-averaged difference in mean arterial pressure was 0.1 mm Hg in favor of atenolol, which was not statistically significant.

Atherothrombotic stroke was the most common form of stroke observed in the LIFE study, and the effects of losartan were greatest for this type of stroke.[6] According to secondary analyses of LIFE data, patients who received a losartan-based treatment had significant reductions in the risk of atherothrombotic stroke (hazard ratio [HR], 0.72 [95% CI, 0.59–0.88; P = 0.001]) and fatal stroke (HR, 0.65 [95% CI, 0.43–0.96]; P = 0.032) compared with atenolol-based therapy.[6] Similar risk reductions were observed for hemorrhagic and embolic stroke, but these stroke subtypes were not significantly different between the losartan and atenolol groups.[6] This observation raises several questions. First, does losartan exert antithrombotic actions beyond lowering BP? Second, are these actions dependent on the angiotensin AT_1 receptor? And third, are these actions common to all AIIAs?

Naghavi et al[7,8] proposed 3 factors as possible contributors to atherothrombotic events: plaques vulnerable to rupture, myocardium vulnerable to fatal arrhythmias, and blood prone to inflammation and thrombosis. Potentially, these factors may be targets of losartan's action. The purpose of this review was to examine the molecular and pharmacologic mechanisms that may be associated with the different outcomes observed in the LIFE study.

METHODS

A PubMed/MEDLINE search of English-language articles (1990 to February 2006) was used to identify possible pharmacologic mechanisms of action. Searches were conducted by combining the terms *angiotensin II antagonists* or *AIIAs* or *angiotensin receptor blockers* or *losartan* or *atenolol* or *beta blocker* with terms including, but not limited to, *atherosclerosis*, *left ventricular hypertrophy*, *carotid artery hypertrophy*, *fatty streaks*, *atrial fibrillation*, *arrhythmias*, *endothelial function*, *myocyte hypertrophy*, *myocardial fibrosis*, *platelet aggregation*, *tissue factor*, *plasminogen activator inhibitor-1*, *PAI-1*, *anti-inflammatory*, *uric acid*, or *oxidative stress*. Articles describing pathophysiologic effects of angiotensin II (AII) and molecular mechanisms of pharmacologic action of losartan pertinent to the LIFE study were, in the opinion of this author, appropriate for review.

LOSARTAN AND VULNERABLE PLAQUE

Atherosclerosis can be viewed as a specialized type of chronic inflammation.[9] Atherogenic factors promote the attachment and proliferation of mononuclear cells on the luminal surface of the arterial wall; these cells eventually migrate across the endothelium and accumulate in the intima.[10] In the presence of oxidized low-density lipoprotein cholesterol (LDL-C), monocytes are converted to activated macrophages and take up lipoprotein particles, resulting in their conversion to foam cells.[11] The continued formation of foam cells and their accumulation in the intima, along with the migration of smooth muscle cells, lead to the development of a fatty streak.[9] The fatty streak progresses to fibrofatty and fibrous plaques, which are characterized by recruitment of extracellular matrix components

and increased deposition of intracellular and extracellular lipid.[9] In the experience of this author, unstable plaques (which are prone to rupture) are characterized by a thin fibrous cap, a high foam-cell content, and expression of various metalloproteinases.

Endothelial Dysfunction

Endothelial dysfunction is an early feature of atherosclerosis and contributes to the atherosclerotic process by promoting abnormal vasomotion, infiltration of inflammatory cells, and increased coagulation.[12] AII promotes endothelial dysfunction through several mechanisms, one of which is inactivation of nitric oxide.[13] In a study of 25 patients with atherosclerotic disease, a 20-minute infusion of losartan 250 μg/min improved coronary vasomotion during flow-mediated dilation in response to adenosine from a mean (SD) of 5.6% (1.5%) to 8.9% (1.8%) ($P = 0.02$).[14] Similarly, in 31 patients with atherosclerosis, treatment with oral losartan 25 to 50 mg for 8 weeks improved brachial artery vasodilation (mean [SD], 1.4% [0.9%] to 3.2% [0.8%]; $P = 0.03$) and significantly increased serum nitrogen oxide levels (mean [SD], 21.6 [1.7] to 26.7 [2.4] μmol/L; $P = 0.008$).[15] Six weeks of treatment with candesartan 16 mg/d has also been associated with an improved forearm blood flow response during reactive hyperemia in a randomized, double-blind study of 47 hypercholesterolemic patients with LDL-C >160 mg/dL (approximate change, 31% vs 5% with placebo; $P < 0.05$).[16] A similar improvement in flow-mediated dilator response to hyperemia with candesartan 16 mg/d for 2 months was noted in a randomized, double-blind, placebo-controlled crossover study in 45 patients with mild to moderate hypertension.[17] Compared with placebo, candesartan was associated with a significant increase in flow-mediated dilator response from a mean (SD) of 5.17% (0.24%) at baseline to 6.22% (0.26%) at the end of 2 months ($P < 0.001$).[17]

It seems possible that another mechanism by which AII may promote endothelial dysfunction is through augmented expression of endothelial adhesion molecules and, in turn, stimulation of leukocyte adhesion to endothelial cells. For instance, it has been reported that AII upregulates intercellular adhesion molecule (ICAM-1) expression by cultured human vascular endothelial cells in vitro derived from umbilical cord veins and stimulates in vitro and in vivo soluble ICAM-1 release.[18] In a separate series of experiments involving patients with essential hypertension, treatment for 4 weeks with losartan 50 mg/d (**Figure 1**, n = 5), but not treatment with atenolol 50 mg QD (n = 4 [data not shown in figure]), was found to blunt the ICAM-1 response to AII.[18]

An early event in atherosclerosis is hypertrophy of the arterial wall,[19] and a reliable surrogate measurement is carotid intima-media thickness.[20,21] In rabbits fed high-cholesterol diets, administration of losartan (25 mg/d for 10 weeks) was associated with significant reductions in intimal proliferation and the ratio of intima to media in the aorta (both, $P < 0.01$ vs high-cholesterol diet alone).[22] In hypertensive patients who were treated for 1 year with losartan, significant reductions in mean (SD) media width to lumen diameter of gluteal arteries (from 8.4% [0.4%] at baseline to 6.7% [0.3%] at 1 year; $P < 0.01$) were observed, whereas treatment with atenolol was not associated with a significant change (from 8.3% [0.3%] at baseline to 8.8% [0.5%] at the end of treatment).[23] The study involved obtaining gluteal subcutaneous biopsies under local anesthesia from 19 patients randomly assigned to treatment with 50 mg of losartan or atenolol in a double-blind fashion. Similar improvements in vascular structure in hypertensive patients have also been reported with irbesartan.[24]

Vascular Oxidative Stress

Atherosclerosis is initiated and amplified by vascular oxidative stress.[25] AII has been implicated in this process through a variety of mechanisms involving alteration of lipid synthesis, lipid oxidation, and lipid entry into susceptible endothelium.

AII (10^{-7} M for 18 hours at 37°C) has been shown to increase in vitro cellular cholesterol biosynthesis (measured by ^{3}H acetate incorporation into cholesterol) by 44%, 34%, and 30%, respectively, in human monocyte–derived macrophages, in mouse peritoneal macrophages, and in J-774A.1 macrophage-like cell line.[26] This effect can be completely attenuated by losartan at a concentration of 10^{-5} M.[26] In addition, AII enhances LDL-C oxidation by stimulating the activity of nicotinamide adenine dinucleotide phosphate oxidases in vascular smooth muscle cells (VSMCs) and endothelial cells.[27–29] In animal models of atherosclerosis, losartan was associated with a significant attenuation of aortic atherosclerotic lesions ($P = 0.001$),[30] a significant reduction in LDL-C oxidation in apolipoprotein(E)-deficient mice after 10 weeks of treatment ($P < 0.005$ vs placebo),[31] and a significant

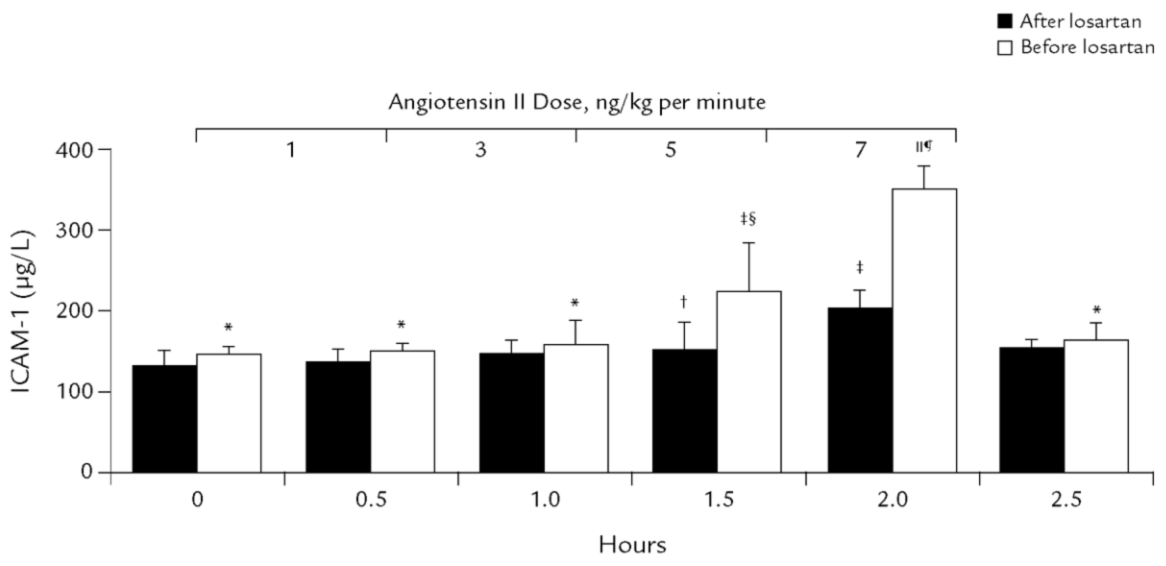

Figure 1. Effect of angiotensin II infusion (at an initial rate of 1 ng/kg per minute, increased every 30 minutes by 2 ng/kg per minute) on circulating intercellular adhesion molecule (ICAM-1) levels in patients with essential hypertension (n = 5) before and after 4 weeks on placebo or losartan 50 mg/d. *$P < 0.05$ versus baseline; †$P < 0.003$ versus time; ‡$P < 0.002$ versus time 0; §$P < 0.003$ versus baseline; ‖$P < 0.0001$ versus time 0; ¶$P < 0.0001$ versus baseline. Reproduced with permission.[18]

reduction in the susceptibility of LDL-C to in vitro oxidation ($P < 0.05$) in hypercholesterolemic monkeys at the end of 6 weeks of treatment.[32] In a randomized, prospective, crossover study, hypertensive patients (n = 40) with type 2 diabetes who received losartan 50 mg/d for 16 weeks experienced significant reductions in LDL-C oxidation (determined by malondialdehyde [MDA] with the thiobarbituric acid–reactive substances assay with and without cupric sulfate) after 16 weeks ($P = 0.001$).[33] Values for LDL-oxidation with cupric sulfate decreased from 40.3 nmol MDA/mg protein at baseline to 34.6 nmol MDA/mg protein after losartan ($P = 0.001$).

Lectin-like oxidized low-density lipoprotein (Ox-LDL) receptor (LOX-1) expression is upregulated by AII in cultured human coronary artery endothelial cells and in primary cultures of human umbilical vein endothelial cells.[34,35] In hypercholesterolemic rabbits, losartan 25 mg/kg daily for 10 weeks was associated with attenuated aortic intimal proliferation ($P < 0.01$) and markedly decreased LOX-1 messenger RNA (mRNA, $P < 0.01$), and LOX-1 expression determined immunohistochemically (no statistical comparison given).[36] In addition, losartan 50 mg/d for 4 weeks was associated with Ox-LDL uptake, as indicated by a 78% decrease in Ox-LDL cell association and a 21% reduction in Ox-LDL degradation, as well as a reduction in the expression of the Ox-LDL receptor CD36 by 54%.[37]

Collectively, the above studies indicate a key role for AII in the initiation and magnification of the atherosclerotic process. Although likely, it is not clear whether AII's effects on this process are mediated by the AT_1 receptor or whether other AIIAs have mechanisms of action similar to those of losartan.

LOSARTAN AND VULNERABLE MYOCARDIUM

It is common knowledge that cardiac arrhythmias occur frequently in hypertensive patients, and that the presence and complexity of arrhythmias may influence the morbidity and mortality of patients, especially those in relation to the occurrence of stroke. Along with diastolic dysfunction of the left ventricle and changes in left atrial size and function, LVH emerges as the main risk factor for arrhythmias in patients with hypertension.[38] In fact, it has been shown that LVH is

associated with proarrhythmic repolarization changes and increased occurrence of atrial fibrillation and ventricular arrhythmias.[39–41]

Myocardial Fibrosis

LVH is characterized by increased extracellular matrix deposition (ie, myocardial fibrosis) and increased myocyte size (ie, myocyte hypertrophy). Myocardial fibrosis results from increased accumulation of fibrillar collagen types I and III throughout the septum and free wall of the left ventricle[42–44] and is thought to promote abnormalities of cardiac function, coronary reserve, and electrical activity.[45] In particular, it has been reported that hypertensive patients with LVH and arrhythmias exhibited higher myocardial collagen tissue deposition and left ventricular mass than patients without arrhythmias.[45]

Various lines of evidence suggest that systemically produced AII, locally produced AII, or both may participate in the development of myocyte hypertrophy and myocardial fibrosis that accompany hypertension via activation of the AT_1 receptor in cardiac myocytes[46] and fibroblasts.[47] Therefore, losartan might exert an antiarrhythmic effect by inducing regression of LVH and reduction of myocardial fibrosis. This is supported by the fact that significant reductions in LVH, myocardial fibrosis, or both have been reported to occur with losartan in hypertensive patients,[48–53] hypertensive animals,[54–56] rat models of hypertension,[57–59] and animal models of MI (all, $P < 0.05$).[60–64]

Further studies have found that these effects were evident at doses ineffective for reducing BP[65] and in animals in which BP was not normalized.[66,67] This effect of losartan was reproduced in hypertensive patients in whom reduction of collagen synthesis and myocardial fibrosis was associated with decreased myocardial stiffness (**Figure 2**).[51,68] Additional support came from the LIFE study, in which all patients had documented LVH. Compared with atenolol-treated patients, patients who received losartan had significantly greater reductions in left ventricular mass index (LVMI) (–21.7 [21.8] vs –17.7 [19.6] g/m^2; $P = 0.021$) and LVH as determined by Sokolow-Lyon voltage on electrocardiogram (–2.5 vs –0.7 mm; $P < 0.001$) or Cornell voltage-duration product (–200 vs –69 mm · ms; $P < 0.001$), despite similar reductions in BP.[69,70]

Other AIIAs have shown similar actions to losartan with regard to reduction of myocardial fibrosis.[71–78] Valsartan has been reported to inhibit myocardial fibrosis (interstitial collagen content) in post-MI rats

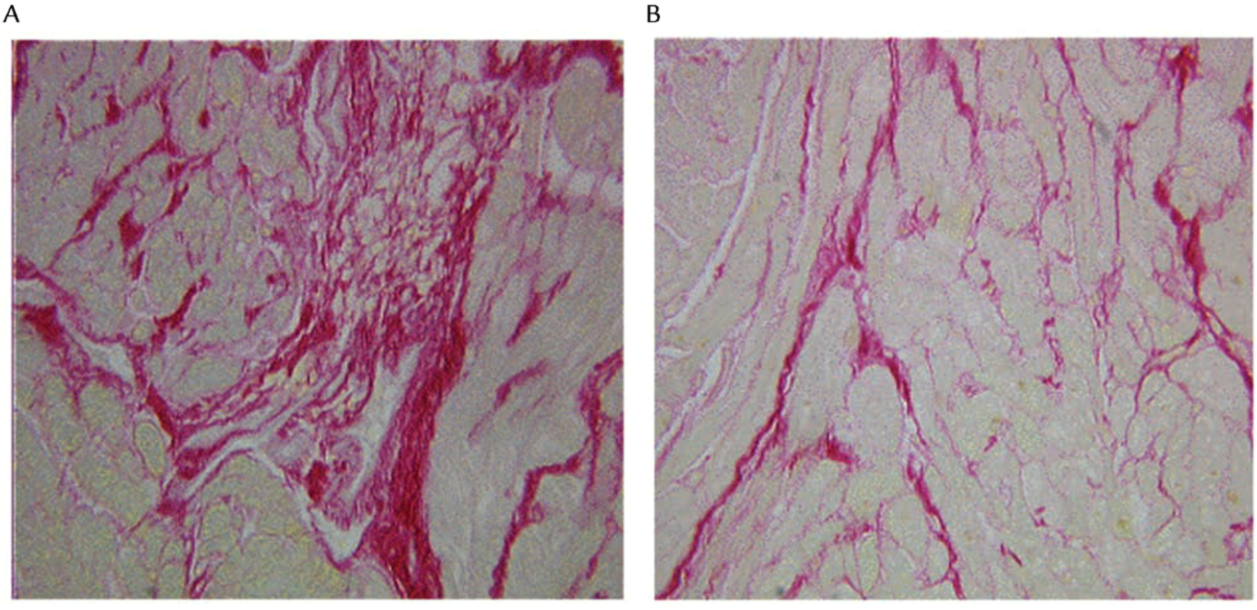

Figure 2. Histologic section of myocardial specimen biopsy from a hypertensive patient with severe myocardial fibrosis. The image used picrosirius red stain and 20× magnification. (A) Before chronic treatment with losartan. (B) After losartan.

($P < 0.05$ vs placebo)[71]; however, the role of BP changes is unclear. Similarly, candesartan has been reported to reduce collagen synthesis in cell culture[79] and to reduce myocardial fibrosis in animal models.[73–78] Although evidence for the action of candesartan or valsartan in humans is limited, a randomized, double-blind study comparing valsartan with atenolol found that treatment with valsartan was associated with significant regression of LVH in largely untreated patients with hypertension.[80] Patients who received valsartan exhibited a decrease in mean (SD) LVMI (from 127 [23] g/m^2 at baseline to 106 [25] g/m^2 at 8 months; $P < 0.001$). Similarly, atenolol-treated patients experienced a significant decrease in mean (SD) LVMI (from 127 [25] g/m^2 at baseline to 117 [27] g/m^2 at 8 months; $P < 0.009$).

Alternative Mechanisms

The arrhythmogenicity of AII may be mediated through alternative mechanisms. In fact, in guinea-pig isolated atria, AII increased norepinephrine release induced by electrical field stimulation (55% maximal augmentation at 10^{-6} mol/L AII), and the effects of 10^{-6} mol/L and 10^{-7} mol/L AII were completely inhibited by 10^{-6} mol/L losartan.[81] Thus, given the evidence that norepinephrine may have proarrhythmogenic effects,[82] it is possible that losartan may have antiarrhythmic properties via inhibition of norepinephrine release in cardiac sympathetic nerves.

Preliminary experimental in vitro data obtained using guinea-pig ventricle and isolated guinea-pig ventricular myocytes suggest that losartan inhibits cardiac delayed rectifier K^+ currents, thus providing a molecular basis for the modification of the atrial and ventricular action potentials.[83] This property may have relevance when considering the antiarrhythmic properties of losartan, given the evidence that losartan reduces QT dispersion, which is an electrocardiographic measure of ventricular repolarization and is also a risk marker for ventricular tachyarrhythmias.[84] This study analyzed QT intervals (calculated by a single, blinded investigator using standard 12-lead electrocardiography) in a total of 90 heart-failure patients at baseline and after active treatment with losartan (n = 47) or captopril (n = 43). QT dispersion (QT_d, defined as the difference between maximum and minimum QT intervals [ie, beginning of the QRS complex to end of the T wave]) increased in patients receiving captopril (from mean [SD] 64 [25] ms at baseline to 74 [25] ms at end of study; $P = 0.008$) but not in patients receiving losartan (from mean [SD] 60 [21] ms at baseline to 60 [22] ms at end of study; P = NS). The difference between groups was significant ($P = 0.01$).

LOSARTAN AND BLOOD VULNERABILITY

Inflammation

Rupture of the sclerotic plaque and the associated formation of thrombus are the most important mechanisms leading to complications associated with atherosclerosis.[85] AII promotes inflammation via induction of nuclear translocation of nuclear factor κ B (NF-κB), thereby upregulating expression of inflammatory cytokines in monocytes, endothelial cells, and VSMCs.[86,87] In aortic segments from hypercholesterolemic rabbits, losartan was associated with significantly reduced activation of NF-κB compared with a control group on a high-cholesterol diet ($P < 0.05$ [n = 4 in each group]); it was postulated that this might have been the result of inhibiting degradation of its inhibitor, Iκ-Bα.[88] In addition to upregulating NF-κB, AII induces monocyte chemotaxis by upregulating monocyte chemoattractant protein 1 (MCP-1) and C-C chemokine receptor 2 (MCP-1 receptor).[89–91] Losartan has been reported to completely inhibit the binding of monocytes to human aortic endothelial cells induced by AII in culture ($P < 0.05$).[92] In addition, losartan inhibits basal MCP-1 expression,[93,94] as well as LDL-C–stimulated MCP-1 expression.[93] Losartan therapy reduced circulating levels of MCP-1 in hypercholesterolemic monkeys after 6 weeks (from ~105 to 70 ng/mL; $P < 0.05$).[32] Eighteen healthy volunteers who received losartan (50 mg/d for 6 weeks) in a randomized, double-blind, crossover study experienced a significant reduction in MCP-1 concentration (from 560 to 423 ng/mL at week 6; $P < 0.01$).[95] Similar actions of candesartan and irbesartan have been reported in hypertensive patients or healthy volunteers,[17,96] and would suggest that some of the actions of losartan on macrophage chemotaxis may be mediated by the AT_1 receptor and common to the AIIAs.

Arachidonic acid metabolism plays an important role in acute ischemic syndromes affecting the coronary or cerebrovascular territory, as reflected by biochemical measurements of eicosanoid biosynthesis and the results of inhibitor trials in these settings.[97] In particular, the clinical efficacy of low-dose aspirin in reducing the short-term complications of acute MI and acute ischemic stroke, as well as in preventing vascular recurrences, has focused attention on the cyclooxygenase

(COX) pathway of arachidonic acid metabolism and its bioactive products.[98] Experimental and clinical tools developed during the past 10 years have allowed one to hypothesize a role for COX-2–derived prostanoids and matrix metalloproteinases in atherosclerotic disease progression and its thrombotic complications.[99]

AII has been found to upregulate COX-2 mRNA in vitro in rat VSMCs,[100] thereby increasing prostaglandin synthesis, an effect that was completely blocked by losartan.[100] Similarly, AII upregulates the expression of both COX-2 mRNA (6.5-fold increase at 3 hours; $P < 0.001$) and protein and stimulates the production of COX-2–dependent prostaglandins in cultured human aortic VSMCs in vitro.[101] Pretreatment of VSMCs with losartan (1 μM for 30 minutes) in vitro attenuated the effects of AII on the expression of the COX-2 protein (without altering the expression of COX-1) and also inhibited the AII-stimulated release of prostaglandin E_2 (PGE_2).[101] A study in patients with symptomatic carotid artery stenosis treated with irbesartan or chlorthalidone for 4 months before endarterectomy has also reported that irbesartan was associated with a decrease in inflammation and inhibition of COX-2/PGE_2-dependent synthase expression in atherosclerotic plaque.[102] The authors suggested that this effect may contribute to plaque stabilization.

Thrombosis

Tissue factor (TF) initiates coagulation through binding to factor VII.[103] In situ hybridization and immunohistochemistry techniques (using a specific riboprobe for TF mRNA and a polyclonal antibody directed against human TF protein) have identified TF-producing cells in normal human vessels and atherosclerotic plaques.[104] Wilcox et al[104] reported the absence of TF mRNA and protein in endothelial cells lining normal internal mammary artery and saphenous vein samples, although TF was synthesized in scattered cells present in the tunica media as well as in fibroblast-like adventitial cells surrounding vessels. In contrast, vessels with atherosclerotic plaques contained many cells synthesizing TF mRNA and protein, including macrophages present as foam cells and monocytes adjacent to the cholesterol clefts.[104] AII has been reported to increase TF expression in cultured endothelial cells and VSMCs.[105,106] Losartan has been reported to reduce expression of TF in leukocytes from healthy volunteers[107] and in cultured VSMCs exposed to AII.[108] In addition, several AIIAs (ie, irbesartan, candesartan, valsartan) have been shown to reduce TF expression in hypertensive patients, transgenic rats, or human cultured monocytes.[109–111]

AII also promotes coagulation by inhibiting the fibrinolytic system by inducing plasminogen activator inhibitor-1 (PAI-1) in the vasculature.[112] Conflicting data suggest that losartan may mediate antifibrinolytic activity via reduction of PAI-1 expression and activity,[113–117] and other reports suggest that losartan may inhibit fibrinolysis by alternate actions through prevention of platelet aggregation (**Figure 3**).[118,119] The different observations about PAI-1 activity and expression may reflect the manner in which the analyses were conducted, such as timing of sample collection and duration of treatment.[113] Reduction of PAI-1 levels by candesartan, valsartan, and irbesartan would, however, suggest that AII-mediated inhibition of fibrinolysis may be affected by the AT_1 receptor.[17,109,120,121]

It is widely acknowledged that transformation of arachidonic acid into prostaglandins such as prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and thromboxane A_2 (TXA_2) is associated with increased platelet aggregation. Altered

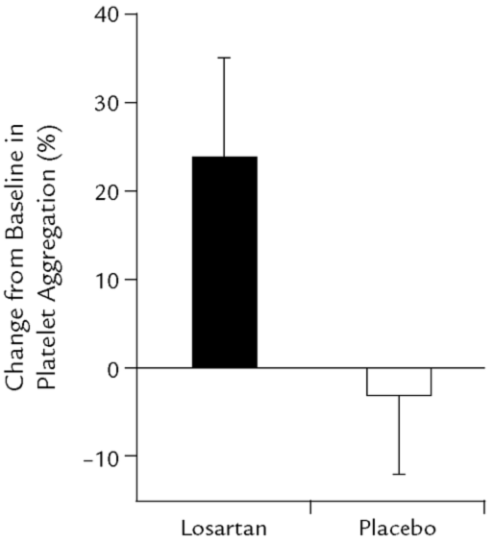

Figure 3. Increase in the extent of platelet aggregation at median effective concentration producing a response of 50% of the maximal response, mediated by a selective thrombin receptor agonist in 9 hypertensive patients administered losartan 50 mg/d or placebo for 4 weeks. Adapted with permission.[119]

platelet function plays a critical role in the development of thrombi. TXA_2, the main product of platelet arachidonic acid metabolism, is a potent inducer of platelet aggregability.[122] Platelet shape change is an early phase of platelet activation that precedes platelet aggregation. AII has been found to induce platelet shape change in human platelets from healthy volunteers, as suggested by a significant increase in median platelet volume ($P < 0.004$).[123] In ~57% of subjects (n = 8) who experienced a marked increase in median platelet volume (>0.4 fL), losartan significantly inhibited platelet shape change induced by AII (from 5.85 fL with AII alone to 5.55 fL after AII plus losartan after 10 minutes; $P < 0.008$).[123] Similar changes were also induced by the TXA_2 analogue U46619.[123] Furthermore, several studies have reported the inhibition of U46619-induced platelet aggregability by losartan in human, canine, or rat platelets in vitro.[124–131] The inhibitory effect of losartan on platelet aggregability appears to be greater with the parent compound than with its metabolite EXP3174.[124,126,127] Losartan-dependent inhibition of $PGF_{2\alpha}$ formation has also been reported in hypertensive patients after oral administration of losartan 100 mg (**Figure 4**).[132] Irbesartan also inhibited the TXA_2 receptor in platelets, but candesartan and valsartan appeared to have little or no effect on platelet aggregability.[128–130]

EMERGING ASPECTS

Serum Uric Acid

Hyperuricemia is common in patients with CV and kidney disease.[133] In the general population, hypertensive patients, and those with preexisting CV disease, serum uric acid (SUA) may represent an important predictor of CV events.[134] However, the link between SUA and risk of CV events is controversial (for detailed review, see Alderman and Aiyer[135]). Studies have reported that SUA is associated with the activation of circulating platelets,[136] upregulation of MCP-1,[137] impaired endothelial nitric oxide release,[138] VSMC proliferation,[139–141] and induction of COX-2, TXA, and other cytokines.[139–142] In a multivariate analysis of patients who had experienced ischemic stroke, high SUA was associated with poor outcome (ie, death or life in care) and with subsequent vascular events (ie, MI, ischemic stroke, or vascular death), based on data from 3731 patients (SUA measurements were available in 2498).[143]

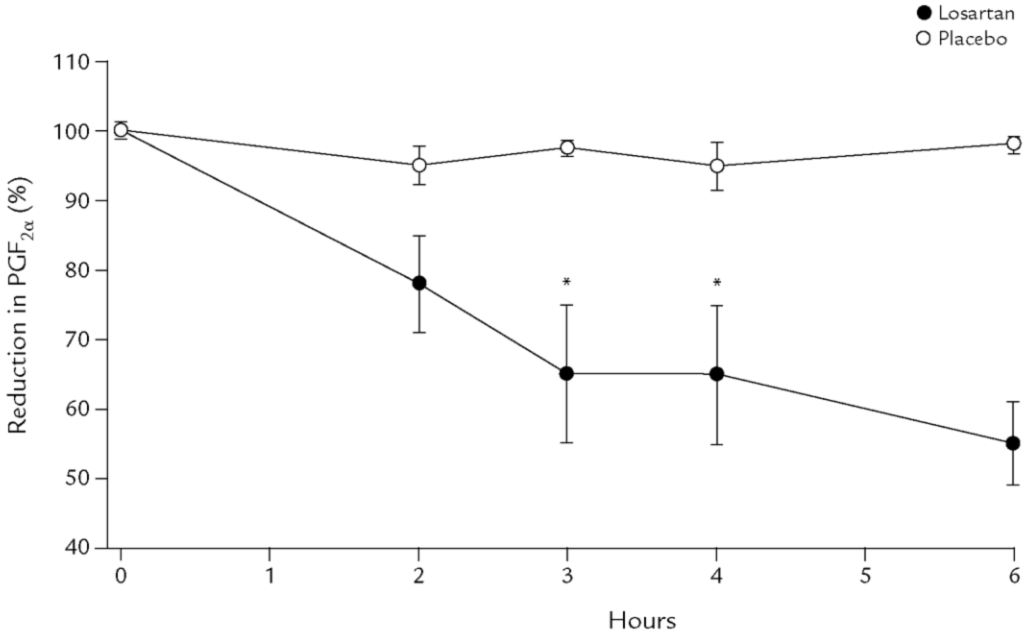

Figure 4. Losartan-dependent inhibition of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) formation in serum samples of 5 patients after oral administration of losartan 100 mg or placebo. $PGF_{2\alpha}$ was measured by enzyme-linked immunosorbent assay. *$P < 0.01$ versus placebo. Reproduced with permission.[132]

Losartan was reported to decrease SUA in healthy Japanese male subjects in a dose-dependent manner, with single doses ranging from 25 to 200 mg.[144] In that study, SUA measured at 4 hours after administering single oral doses of losartan 25, 50, 100, or 200 mg (n = 6), or placebo (n = 3), was decreased from baseline by a mean of 0.32 mg/dL (with losartan 25 mg), 0.77 mg/dL (with losartan 50 mg), 1.25 mg/dL (with losartan 100 mg), and 1.33 mg/dL (with losartan 200 mg).

Losartan has been found to produce a uricosuric effect.[145] Data from Sweet et al[146] suggest that the parent molecule, not its active metabolite EXP3174, is the active agent blocking uric acid absorption. Furthermore, the effect of losartan on SUA does not appear to be shared with other members of the AIIA class.[147–149] Recently, the LIFE study reported a possible association of baseline SUA with MI, stroke, or CV death (HR, 1.024 per 10 µmol/L [95% CI, 1.017–1.032]; $P < 0.001$).[150] Although SUA levels increased in both the losartan and atenolol groups, patients who were treated with losartan had a significant attenuation of the increase in SUA from baseline.[150] The increase from baseline in mean (SD) SUA was greater in atenolol-treated subjects than in losartan-treated subjects (44.4 [72.5] vs 17.0 [69.8] µmol/L; $P < 0.001$). The association of SUA with CV events was significant in women (HR, 1.025 [95% CI, 1.013–1.037]; $P < 0.0001$) and remained so after adjustment for the Framingham risk score (HR, 1.013 [95% CI, 1.00–1.025]; $P < 0.046$).[150] However, the relationship between baseline SUA and CV events was not significant in men (HR, 1.009 [95% CI, 0.998–1.019]). SUA as a time-varying covariate was also associated with events in the entire population ($P < 0.001$); this association was strong in women ($P < 0.001$), but was not present in men (P = NS).[150] The underlying reasons for this apparent gender difference are unclear, however.

The Losartan Metabolite EXP3179

Given the differences in the effects of losartan on platelet aggregability, compared with other AIIAs, losartan's metabolite has been identified and its effects on COX-2 and its role in platelet aggregability investigated.[132] Losartan is metabolized by the liver primarily by the cytochrome P450 (CYP) 2C (CYP2C) enzymes, with some additional involvement of the CYP3A enzymes (**Figure 5**).[151] Losartan is biotransformed to the active carboxylic acid derivative EXP3174, which has been shown to be 40 times more potent at blocking the AT_1 receptor than losartan.[152] EXP3179, an intermediate aldehyde compound in the metabolism of losartan, has also been identified[151] and reported to have extremely low (if any) affinity for the AT_1 receptor.[132] Plasma levels of EXP3179 after administration of losartan have been reported to increase at 2 hours and peak at 3 to 5 hours before disappearing at ~6 hours postadministration.[132]

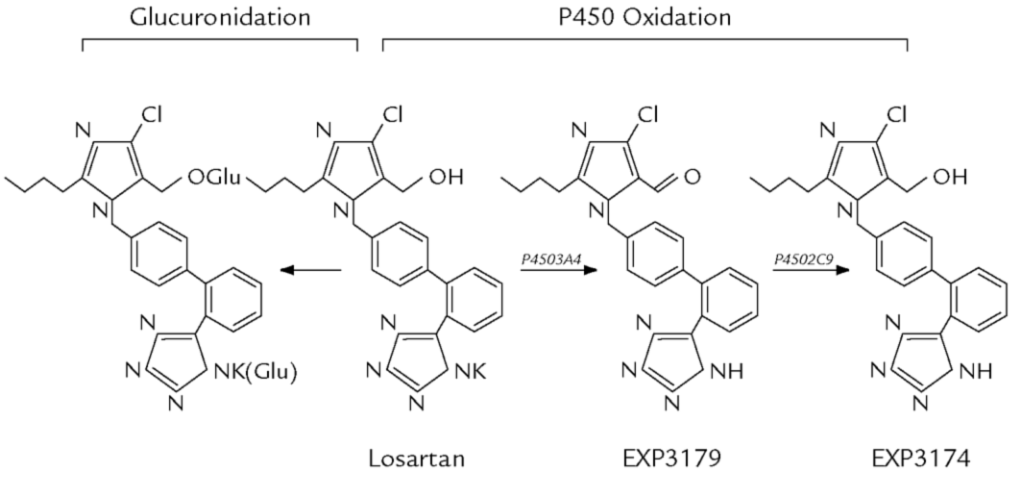

Figure 5. Schema for the cytochrome P450 oxidative biotransformation of losartan to its active carboxylic acid metabolite, EXP3174, and its aldehyde intermediate, EXP3179.

Recently, Kramer et al[132] reported that EXP3179 had anti-inflammatory activities that were independent of the AT_1 receptor and were mediated by regulation of COX-2 mRNA expression. EXP3179 was thought to elicit COX-2 inhibitory properties by blocking COX-2 mRNA upregulation and $PGF_{2\alpha}$ synthesis induced by AII and bacterial lipopolysaccharide (LPS), an AT_1 receptor-independent proinflammatory stimulus, in human endothelial cells.[132] EXP3179 also prevented upregulation of ICAM-1 induced by LPS in these cells.[132] Finally, EXP3179 abolished platelet aggregation induced by arachidonic acid and U46619.[132] Importantly, treatment of hypertensive patients with a single dose of losartan 100 mg resulted in a decrease in platelet aggregability at ~6 to 8 hours after administration, which coincided with peak plasma concentrations of EXP3179.[132] The anti-inflammatory and antiaggregatory effects of losartan appear to be partially independent of its blockade of the AT_1 receptor and may be related to alternative mechanisms elicited by its metabolite, EXP3179.

Research using the endothelium of intact rat aorta and endothelial cells in vitro has found that EXP3179 stimulates endothelial nitric oxide synthase (eNOS) phosphorylation and suppresses tumor necrosis factor-α–induced endothelial cell apoptosis by activating the vascular endothelial growth factor receptor-2 signaling pathway.[153] The magnitude for EXP3179-stimulated phosphorylation of eNOS was much greater than that of EXP3174, and the median effective concentration producing a response of 50% of the maximal response (EC50) was significantly lower (mean [SD] –logEC50, 8.2 [0.1] vs 5.4 [0.2] mol/L, respectively), suggesting an AT_1 receptor–independent beneficial effect of the losartan metabolite EXP3179 on the endothelium.

Finally, a study identified EXP3179 as a partial peroxisome proliferator–activated receptor γ (PPAR-γ) agonist.[154] In fact, EXP3179 was found to promote 3T3-L1 adipocyte differentiation, induce PPAR-γ target gene expression, and directly activate the PPAR-γ–ligand-binding domain (LBD). EXP3174 did not induce PPAR-γ–LBD activation and failed to regulate adipocyte differentiation of PPAR-γ target gene expression.

EFFECTS OF ATENOLOL: INTERPRETATION OF LIFE STUDY DATA

Although the results of the LIFE study may have been due to a possible benefit of losartan other than BP control, an alternative interpretation of the LIFE data has also been put forth: the results could be partly due to lack of benefit of atenolol. In this regard, a meta-analysis of randomized trials has suggested that β-blockers (and specifically atenolol) may not provide equivalent reduction in CV events compared with other antihypertensive agents.[155] The recent meta-analysis by Carlberg et al[155] disputed the choice of atenolol as an appropriate comparator in clinical trials involving patients with hypertension. That analysis focused on 4 randomized trials that compared atenolol with no treatment and 5 trials that compared atenolol with other antihypertensive drugs in hypertensive patients. In the studies comparing atenolol and placebo, no differences in outcome were apparent with respect to all-cause mortality (relative risk [RR], 1.01), cardiovascular mortality (RR, 0.99), or MI (RR, 0.99); stroke risk appeared to be slightly lower in the atenolol group (RR, 0.85), but this was not statistically significant. In 5 studies comparing atenolol with other antihypertensives (HCTZ/bendroflumethiazide, captopril, losartan, or lacidipine; 17,671 patients followed over a mean of 4.6 years), a significantly higher risk of mortality (RR, 1.13 [95% CI 1.02–1.25]) was found to occur with atenolol compared with the other active treatments.

These results have been challenged by a separate meta-analysis conducted by Edelman et al.[156] In their meta-analysis of 6 randomized outcomes trials, a β-blocker (including atenolol-based studies, other β-blocker studies [ie, propranolol alone, atenolol/metoprolol/pindolol combined], and diuretic-based studies in which atenolol was used as add-on therapy) was associated with a 26% risk reduction in all CV events ($P < 0.001$), a 19% risk reduction in CV death ($P = 0.001$), a 34% risk reduction in stroke ($P < 0.001$), and a 20% risk reduction in coronary heart disease ($P < 0.001$), compared with placebo or no treatment. The investigators suggested that a possible reason for the differences in findings between the 2 analyses may involve the types of study selected in each case. In this regard, it is important to note that in the meta-analysis by Carlberg et al,[155] 2 of the 4 studies evaluated the use of atenolol for secondary (not primary) prevention of stroke and secondary cerebroprotection.

Another possible explanation for the difference between stroke risk with losartan and atenolol observed in LIFE may involve differences in central pulse pressures between groups.[157] According to results from the Anglo-Scandinavian Cardiac Outcomes Trial Conduit

Artery Function Evaluation (ASCOT CAFE) study in hypertensive patients,[158] central aortic pressures may be a better predictor of events such as stroke and MI than conventional brachial BP. ASCOT CAFE was a substudy of ASCOT and measured central and brachial BPs in 2073 hypertensive patients receiving either an amlodipine/perindopril regimen or an atenolol/bendroflumethiazide regimen. The CAFE study found that central aortic pulse pressure was 3 mm Hg lower in the amlodipine-based regimen than in the atenolol-based regimen ($P < 0.001$), whereas brachial pulse pressure was slightly higher (0.9 mm Hg) but not significantly different. Although these findings suggest that BP-lowering agents may have different effects on central aortic pressures, which may, in turn, translate to different clinical outcomes, it must be borne in mind that the brachial BPs were well controlled but not identical between study groups. The mean brachial diastolic BP was found to be 1.6 mm Hg lower in patients randomized to amlodipine ($P < 0.001$). Although differences in central aortic pressures may have contributed to the stroke benefit observed in the LIFE study,[159] central BP measurements were not part of the study methods. Thus, the concern cannot be confirmed or refuted.

CONCLUSIONS

A losartan-based regimen was associated with a significantly lower rate of stroke than was atenolol in hypertensive patients with LVH in the LIFE study, despite similar BP reductions. This review of the literature suggests that losartan (and perhaps other AIIAs) may possess a number of properties, independent of its antihypertensive effects, that may be associated with decreased vulnerability of the plaque, myocardium, and blood. Although some of these effects may be shared by other AIIAs or other antihypertensive classes (eg, angiotensin-converting enzyme inhibitors), some of losartan's effects appear to be molecule specific.

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