Original Article

Combined Treatment with an AT1 Receptor Blocker and Angiotensin Converting Enzyme Inhibitor Has an Additive Effect on Inhibiting Neointima Formation via Improvement of Nitric Oxide Production and Suppression of Oxidative Stress

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Accumulating evidence shows that inhibition of the vascular renin-angiotensin system results in suppression of injury-elicited neointima formation. We attempted to determine whether or not combined treatment with an angiotensin II type 1 receptor blocker (ARB) and angiotensin converting enzyme inhibitor (ACEI) has an additive inhibitory effect on balloon-injury-elicited neointima formation in the carotid artery. Male Sprague-Dawley rats were treated with an ARB (valsartan: 3 mg/kg/day) and/or an ACEI (benazepril: 0.3 mg/kg/day) from 1 week before until 2 weeks after balloon injury. Experiments were also conducted with one-third of the dose combination used in the original experiments. Both ARB and ACEI inhibited neointima formation without any blood pressure changes. The full-dose combination lowered blood pressure and suppressed neointima formation significantly compared with the levels in the groups treated with either ACEI or ARB alone. The low-dose combination without blood pressure reduction also inhibited neointima formation to a similar extent as the full-dose combination. We measured 8-iso-prostaglandin F2α (8-iso-PGF2α), a marker of oxidative stress, and nitrite and nitrate (NOx), an index of nitric monoxide production, in media conditioned by the injured artery. NOx production was lower and 8-iso-PGF2α was higher in the media of the injured artery, compared with those in the normal artery. ACEI restored NOx production more dramatically than ARB, and ARB suppressed 8-iso-PGF2α markedly compared with ACEI. These results suggest that the combination of an ARB and an ACEI exerts an additive inhibitory effect, presumably through an increase in production and bioavailability of NO from the endothelium. (Hypertens Res 2004; 27: 129–135)

Key Words: neointima formation, angiotensin II receptor blocker, angiotensin converting enzyme inhibitor, 8-iso-prostaglandin F2α, nitric oxide

Introduction

The renin-angiotensin (RA) system has been shown to be an important blood pressure-regulating system based on the results of many animal experiments as well as the clinical effects of angiotensin-converting enzyme (ACE) inhibitors (1, 2) and angiotensin II receptor blockers (ARB) (3, 4), and has been shown to play a pivotal role in cardiovascular remodeling in patients with conditions such as cardiac hypertrophy, thickening of the vessel walls and atherosclerosis. ARB and ACE inhibitors have an inhibitory effect on the thickening...
of the vessel walls associated with hypertension, and this effect is not dependent on the concentration of circulating angiotensin (Ang) II or the degree of decrease in blood pressure, which suggests that the local vascular RA system is important in this process. Recent studies have shown that angiotensinogen (5), renin and ACE, which are components of the RA system, are produced in the vessel walls. It has also been reported that chymase, an Ang II-producing enzyme that is not an ACE, is present in the local RA system at particularly high concentrations in humans.

In animal models of hypertension and patients with hypertension, the local vascular RA system is activated by pressure exerted on the vessel, which leads to thickening of the vessel walls. It has been reported that there are more Ang II type 1 (AT1) receptors in thickened vessels in spontaneously hypertensive rats (6). Increases in the production of Ang II have been shown to elicit the production of free radicals in monocytes and macrophages that have migrated to the vessel walls resulting in injury to endothelial cells. It is known that, when cultured vascular smooth muscle cells are exposed to Ang II, protein synthesis through AT1 receptors is accelerated and NADH/NADPH oxidase is activated, which increases oxidative stress (7). In atherosclerosis and hypertension, production of nitrogen monoxide (NO), which suppresses cell proliferation, is reduced, and vascular remodeling occurs through local increases in AT1 receptors in response to suppressed NO production (8). It has also been reported that proliferation of coronary artery endothelial cells is suppressed through the action of the Ang II type 2 (AT2) receptor (9). In experimental animals with arterial balloon injury, ACE activity and the expression of AT1 receptors are increased in the vessel walls, and a neointima is formed by migration and proliferation of the medial smooth muscle cells. These changes are inhibited by ACE inhibitors (10) or ARBs (11, 12). These agents are both known to suppress thickening of the vascular intima: ARB by blocking the action of Ang II through AT1 receptors and ACE inhibitors by inhibiting the production of Ang II. Since ARBs and ACE inhibitors suppress the RA system by pharmacologically different mechanisms, it is assumed that the combined use of these agents would have an additive effect in inhibiting the action of Ang II. Recently, the combined use of ARBs and ACE inhibitors has been reported to be helpful for improving cardiac function in pigs and rats with experimental heart failure (13, 14). However, details of the influence of this combination therapy on vascular remodeling and the mechanism of the additive effect are not clear. We investigated the effects of combined treatment with an ARB and ACE inhibitor on vascular neointima formation and the mechanism(s) of the additive effect in a rat vascular endothelial injury model.

Methods

Ten-week-old Sprague-Dawley rats were obtained from Charles River Japan (Tokyo, Japan). Valsartan and benazepril were supplied by Novartis Pharmaceutical (Basel, Switzerland). The rats were divided into five groups (n = 6 each). Blood pressure and heart rate were measured at 1 week before and 2 weeks after the balloon injury by the tail-cuff method (15) using a BP-98A (Softron, Tokyo, Japan). Group 1 (control) rats were not treated with either drug, but received an equivalent dose, subcutaneously, of vehicle only (0.1 eq/l KOH adjusted to pH 7.4 with 1 eq/l HCl) administering using a mini-osmotic pump (Alza, Palo Alto, USA). Group 2 rats were given benazepril orally at 0.3 mg/kg/day and received vehicle alone via a mini-osmotic pump. Group 3 rats were given valsartan at 3 mg/kg/day using a mini-osmotic pump. In Group 4, benazepril and valsartan were administered using the same dosages and routes as in Groups 2 and 3. In Group 5, benazepril (0.1 mg/kg/day) and valsartan (1 mg/kg/day) were administered, i.e., at one-third of the dosages used in Groups 2 and 3. These treatments were given from 1 week before to 2 weeks after the balloon injury. Balloon catheterization of the left common carotid artery was performed on day 0 under anesthesia by sodium pentobarbital (40 mg/kg, i.p.) using a 2F Fogarty balloon catheter (Baxter, Deerfield, USA) at 2 atm (atmosphere), as reported previously (16). After 14 days, the balloon-injured arteries were removed rapidly, cut into 5-mm pieces and embedded in paraffin for histological analysis. Thin sections (4 µm) were cut and stained with hematoxylin and eosin (HE) and Azan-Mallory stains. Neointimal and medial areas were measured by a computer-assisted image analyzer using NIH Image 1.58 software.

To determine oxidative stress and NO production, another group of animals (n = 6 each) were prepared as described above, under sodium pentobarbital anesthesia. After 4 days, the balloon-injured arteries were removed rapidly. In addition, the contralateral normal arteries were obtained from Group 1 and served as controls. These arteries were incubated in 1 ml of Hank’s balanced salt solution under 5% (v/v) CO2/95% (v/v) O2 for 24 h. The contents of 8-iso-prostaglandin F2α (8-iso-PGF2α) and nitrite and nitrate (NOx) in the medium were determined using an enzyme immunoassay kit and NOx colorimetric assay kit (Cayman Chemical, Ann Arbor, USA), respectively.

Statistical Analysis

All data were expressed as the means ± SEM. Differences between the groups were analyzed by ANOVA followed by Scheffe’s test and were considered to be significant when the p value was less than 0.05.

Results

As illustrated in Fig. 1, the systolic blood pressure (SBP) in Group 1 was not significantly different from that 1 week before the balloon injury. The administration of benazepril or valsartan did not lower SBP, whereas concomitant admin-
administration of both drugs significantly lowered blood pressure when compared to that in controls and the groups treated with benazepril or valsartan alone. However, concomitant administration of both drugs at dosages one-third of those used in Groups 2 and 3 did not lower SBP.

Figure 2 shows a typical histological section of the balloon-injured carotid arteries stained with HE. When compared to that in the controls, neointima formation was markedly attenuated in the groups given benazepril or valsartan alone. Concomitant administration of benazepril and valsartan either at the full or one-third dosages inhibited the neointima formation compared with that in rats treated with either benazepril or valsartan alone. The results of the statistical analysis are shown in Fig. 3. Benazepril or valsartan significantly inhibited neointima formation, and this was decreased further by concomitant administration of benazepril and valsartan either at the full or one-third dosages.

As shown in Fig. 4, balloon injury significantly increased the production of 8-iso-PGF\textsubscript{2α} compared with that in controls (the contralateral non-injured artery). Benazepril treatment lowered the augmented 8-iso-PGF\textsubscript{2α} levels significantly. Administration of valsartan also decreased the 8-iso-PGF\textsubscript{2α} levels significantly compared to the levels in Group 2 rats, which were treated with benazepril alone. The combination of benazepril and valsartan at either a full or one-third dose of both drugs decreased the augmented 8-iso-PGF\textsubscript{2α} level significantly compared to that in Group 2 rats. It is of interest that the 8-iso-PGF\textsubscript{2α} levels in Groups 3–5, i.e., the groups treated with ARBs with or without ACE inhibitors, were not different from those of the controls (the contralateral non-injured artery).

NO\textsubscript{x} production by the control and balloon-injured arteries is shown in Fig. 5. Balloon injury resulted in significantly lower NO\textsubscript{x} levels than in the control contralateral non-injured arteries. Treatment with benazepril or valsartan significantly lessened the drop in NO\textsubscript{x} production. However, NO\textsubscript{x} levels in the benazepril-treated group were significantly higher than those in the valsartan-treated group. The combination treatment with benazepril and valsartan at either dosage also lessened the drop in NO\textsubscript{x} levels, and at the full dose of the combined drugs, the NO\textsubscript{x} levels were normalized.

**Discussion**

The present study revealed that the combined use of ACE inhibitors and ARBs had an additive effect on the suppression of vascular remodeling. It is known that, in vascular remodeling in which the endothelium is injured, the RA system is activated, resulting in an increase in ACE activity and expression of AT\textsubscript{1} receptors in the vessel walls, which leads to neointima formation (15, 17). ACE inhibitors and ARBs that have an inhibitory effect on the RA system have been reported to suppress intimal thickening (10, 11). In the present study, the ACE inhibitor and ARB also produced roughly the same degree of inhibitory effect on intimal thickening at dosages that had no blood pressure-lowering effects. Although the inhibitory effect of ACE inhibitors on vascular remodeling was detected in in vivo experiments on rats, such an effect was not observed in humans in the MERCATOR (18) and MARCATOR (19) studies on the prevention of post-PTCA restenosis. This may be due to the fact that vascular remodeling involves Ang II produced by enzymes other than ACE, such as chymase, tonin (20) and cathepsin (21, 22). ACE converts Ang I to Ang II (23) in humans. ARB specifically blocks AT\textsubscript{1} receptors, and thus it blocks the action of Ang II—including that of Ang II produced outside the ACE pathway—at the level of the receptor. Thus, in the recent VAL-PREST trial, ARBs were reported to suppress not only intimal injury in rats, but also post-PTCA restenosis of the coronary arteries in humans (24). Since ACE inhibitors inhibit the production of Ang II while ARBs block AT\textsubscript{1} receptors, including local receptors in the vessels, the combined administration of these two agents can be inferred to exert an additive effect. Indeed, the combined administration of ARB and ACE inhibitor has been reported to have an additive effect on blood pressure lowering (25), reduction of urinary protein (26), and improvement of exercise capacity in patients with severe congestive heart failure (14), as well as an additive anti-atherosclerotic effect, and an additive improvement in the bioavailability of NO in coronary artery disorders (27).

In the present study, the combination of an ACE inhibitor and ARB resulted in a significantly greater suppression of intimal thickening than that by either drug alone. In addition, the combination of an ACE inhibitor and ARB at one-third of the original doses used had no blood pressure-lowering effects and also prevented intimal thickening, indicating that combination therapy with an ACE inhibitor and ARB has an additive effect in suppressing intimal thickening, independent of blood pressure.
To elucidate the mechanisms of their inhibitory actions on neointima formation, the effects of the ACE inhibitor and ARB, singly or in combination, on oxidative stress were compared in the injured vessels. In the injured vessels, the increases in 8-iso-PGF$_2\alpha$ levels in response to injury were decreased significantly by either the ACE inhibitor or ARB, with the ARB inducing the greater decrease. The combination of the two agents at the full dose shown to have a blood pressure lowering effect inhibited the production of 8-iso-PGF$_2\alpha$ slightly, but not significantly, and when the two agents were administered at the one-third dose that elicited no blood pressure change, 8-iso-PGF$_2\alpha$ levels were lowered to the same degree as by ARB alone. In vascular and other local Ang II production, the presence of pathways other than ACE, such as the chymase pathway, has been confirmed.
Kim et al. reported that combination treatment with temocapril (an ACE inhibitor) and CS-866 (an AT1 receptor antagonist) along with blood pressure reduction prevent vascular smooth muscle cell proliferation in the intima to a greater extent than monotherapy with either agent and blood pressure reduction. They also demonstrated that L-NAME, an NO synthase inhibitor, significantly attenuated the diminished intimal hyperplasia induced by combined administration of temocapril and CS-866 (32). In another study, soluble guanylate cyclase α1 and β1 gene transfer to balloon-injured rat carotid arteries increased the NO responsiveness of vascular smooth muscle cells, and reduced neointima formation after balloon injury in rats (33). In another study, however, when an ACE inhibitor and ARB were given together during both myocardial ischemia and reperfusion, myocardial energy metabolism and relaxation were reported to be improved, and this improvement was not deteriorated by L-NAME, suggesting that the combination of an ACE inhibitor and ARB may be effective in patients with cardiovascular disease, but the beneficial effect of NO synthase in such patients is different from the effect of NO synthase in balloon-injured neointimal formation (34).

In agreement with these previous studies, the present study also demonstrated that the ACE inhibitor without a blood pressure lowering effect increased NO production to a greater extent than the ARB. This may have contributed to the additive effect of the two agents, since combination treatment with a full dosage of both drugs resulted in a slightly, but not significantly, higher NO production than treatment with the ACE inhibitor alone.

In summary, the present results clearly showed that the ARB was more effective than the ACE inhibitor in producing an improvement in oxidant stress, and that the ACE inhibitor caused a greater increase in NO production than the ARB in vessels with lesions. The two agents inhibit the RA via different mechanisms, which makes their combined use advantageous. The combination of the effect of ACE inhibitors in improving NO production and the effect of ARBs in suppressing oxidative stress would additively improve the bioavailability of NO in the injured vessels, and this may be one of the reasons why neointimal thickening is suppressed. These findings suggest that combination therapy using an ACE inhibitor and ARB may be effective in preventing the atherosclerosis associated with hypertension.

References


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