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Rapid Communication

Angiotensin-converting Enzyme Inhibitor Versus Angiotensin II, AT_1 Receptor Antagonist

Effects on Smooth Muscle Cell Migration and Proliferation After Balloon Catheter Injury

Margaret Forney Prescott,* Randy L. Webb,* and Michael A. Reidy†

From the Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, Summit, New Jersey, and the Department of Pathology,† University of Washington, Seattle, Washington*

The angiotensin-converting enzyme (ACE) inhibitor, benazeprilat and the angiotensin II (Ang II), AT_1-specific receptor antagonist, DuP753, were compared for their effects on intimal lesion formation as well as smooth muscle cell (SMC) proliferation and migration in Sprague Dawley rats after carotid balloon injury. Both the ACE inhibitor (benazeprilat, 3 mg/kg/day) and the AT_1 antagonist (DuP 753, 10 mg/kg/day) significantly reduced intimal lesion formation after balloon injury (by 35% and 49%, respectively). Medial SMC proliferation after injury was reduced 53% by the AT_1 antagonist; however, the ACE inhibitor had no effect on SMC proliferation. SMC migration was reduced 94% by the AT_1 antagonist and 68% by the ACE inhibitor. These data demonstrate the importance of Ang II in SMC proliferation and migration after balloon injury. They also demonstrate that in the balloon injury model, the ACE inhibitor reduced intimal lesion size by inhibiting SMC migration alone without affecting SMC proliferation. A more pronounced reduction in lesion size was obtained after AT_1 antagonism, however, when both SMC migration and proliferation were inhibited. (Am J Pathol 1991, 139:1291–1296)

This study determined whether an ACE inhibitor, which inhibits the conversion of Ang I to Ang II has the same effect on intimal lesion formation as an Ang II receptor antagonist. Although Ang II interacts with two receptor subtypes, namely AT_1 and AT_2,[1] the AT_1 receptor has been shown to mediate Ang II-induced vascular events.[2,3] Thus the Ang II, AT_1 specific antagonist DuP753[1,2,4] was compared with the ACE inhibitor benazeprilat, the active metabolite of the pro-drug benazepril.

Various ACE inhibitors have been demonstrated to inhibit lesion formation after balloon catheterization.[5] In addition, a recent preliminary report also described lesion inhibition with an Ang II, AT_1 antagonist.[6] However, the mechanism by which lesion formation was inhibited has not been addressed. Intimal lesion formation in the rat balloon injury model occurs by a combination of smooth muscle cell (SMC) migration into the intima and SMC proliferation.[7–9] In the rat, intimal lesion development can be reduced by inhibition of SMC migration alone, in the absence of an effect on SMC proliferation.[10,11] The contribution of SMC migration in development of restenotic lesions in humans after angioplasty, however, is still unclear. The mechanism by which compounds inhibit lesion development (i.e., via inhibition of SMC migration or proliferation or both) must be determined to better understand results of clinical restenosis trials.

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Address reprint requests to Dr. Margaret F. Prescott, Pharmaceuticals Division, Ciba-Geigy Corporation, 556 Morris Avenue, Summit, NJ 07901.

The doses of the two compounds used in the current balloon injury study had previously been determined to produce maximal reductions in blood pressure in spontaneously hypertensive rats (SHR)[12] as well as equipotently inhibit the angiotensin I-induced pressor response.[12] Our balloon injury studies, however, were performed in normotensive Sprague Dawley rats since Ang II does not appear to play a major role in blood pressure regulation in normotensive animals.[12]

Materials and Methods

Blood Pressure Effects and Compound Dose Selection

Blood pressure was measured in male SHR and Sprague Dawley rats (Taconic Farms, Germantown, NY, and Charles River, Wilmington, MA) (n = 7–9) weighing 250–350 g by femoral catheterization, as previously described.[13] Compounds were administered intraperitoneally (IP) via Alzet 2ML2 minipumps (Alza Corp., Palo Alto, CA) implanted subcutaneously. Each minipump was attached to a catheter, which was inserted IP. Alzet pumps were unprimed and catheter lines were filled with saline. Taking into account the length of the catheter lines and the need for approximately 4 hours of priming before the pumps were activated, this procedure resulted in approximately an 8-hour delay between implantation of the pumps and actual delivery of compound IP. Thus pressure measurements at 5 hours were considered to represent baseline readings. Arterial pressure was measured at 5 hours (baseline) and at days 2, 3, and 4. Changes in mean arterial pressure in drug treatment groups were compared with those obtained in vehicle treatment groups. Blood pressure effects after administration of various doses of the ACE inhibitor benazeprilat and the AT_1 antagonist DuP753 were compared to identify doses that lowered mean arterial pressure by 25–30 mm Hg in SHR. The doses identified were 3 mg/kg/day of benazeprilat and 10 mg/kg/day of DuP753. These doses were then tested in Sprague Dawley rats to determine arterial pressure effects in normotensive animals. Both benazeprilat and DuP753 were administered using 1N NaOH as the vehicle.

Lesion Formation Studies

The left common carotid artery was balloon–catheter-injured using Sprague Dawley rats (Taconic Farms) (n = 7–9) weighing 400–450 g as previously described.[9,14] Benazeprilat (3 mg/kg/day in 1N NaOH) and DuP753 (10 mg/kg/day in 1N NaOH) were administered via Alzet 2ML2 minipumps (Alza Corp.) implanted IP. Pumps were implanted 2 days before balloon catheterization to allow high circulating doses to be achieved by the time of vascular injury. Compound administration continued through 12 days postballooning, at which time the animals were sacrificed and the carotids perfusion fixed under pressure as previously described.[15] One-half hour before sacrifice, 0.5 ml 5% Evans Blue was injected intravenously to allow discrimination of re-endothelialized areas at the time of histologic processing.

Two samples from the central blue region of each left carotid and two from the corresponding region of each right carotid were embedded in paraffin. Each block was sectioned (5μ) and sections were stained with orcein. Four cross sections of left carotid were measured for intimal area as previously described.[15] No intimal thickening was present in any of the contralateral control right carotid sections.

SMC Proliferation Studies

Compound administration and balloon catheterization were performed in Sprague Dawley rats as described earlier except that Alzet minipumps were 2ML1. ^{3}H-thymidine (50 μCi/100 gm) was administered at 24, 36, and 47 hours postballoon injury. At 48 hours, animals were sacrificed and perfusion fixed and vessels were embedded and sectioned as described earlier. Autoradiography was performed as described previously[9] and the number of labelled medial SMC were counted and expressed as a percent of total number of medial SMC.

SMC Migration Studies

Compound administration and balloon catheterization were performed in Sprague Dawley rats as described earlier. At 4 days post-balloon injury, animals were sacrificed and perfusion fixed and vessels were embedded and sectioned as described earlier. The number of intimal SMCs was counted in a minimum of four cross sections per block. Since SMC migration into the intima is known to first occur between days 3 and 4 post-injury,[7–9] and, furthermore, since at day 4 intimal SMCs could have undergone only limited SMC proliferation,[9] we therefore considered intimal SMC number at 4 days to represent SMC migration. These studies were performed in two parts for logistical reasons. Groups consisted of four to five animals in each part. Statistical analysis appropriate for a study performed in two parts was chosen.

Statistics

Compound effects were analyzed using one-way ANOVA. An F test for contrast was added to the ANOVA

analysis for the SMC migration study, as that study was performed in two parts.

Results

In SHR, 3 mg/kg of benazeprilat was found to produce a decrease in blood pressure (−25 to −30 mm Hg) equipotent to 10 mg/kg of DuP753 when compared with the NaOH vehicle-treated group. Subsequent blood pressure studies in normotensive Sprague Dawley rats demonstrated that 3 mg/kg of benazeprilat produced a mean decrease of 15 mm Hg compared with control ($P < 0.05$), whereas 10 mg/kg of DuP753 did not lower arterial pressure.

Treatment with the ACE inhibitor benazeprilat and the AT_1 antagonist DuP753 significantly inhibited lesion size at 12 days postballooning (Figure 1). When the degree of lesion inhibition obtained with benazeprilat and DuP753 treatment was compared, no statistically significant difference was found.

Since lesion formation after balloon injury is due to a combination of SMC proliferation and migration, these phenomena were studied in separate experiments. SMC replication was determined between 24 and 47 hours postballoon injury by ^{3}H-thymidine administration and autoradiography. The AT_1 antagonist DuP753 was found to reduce DNA synthesis in medial SMCs by 53%, whereas the ACE inhibitor benazeprilat had no effect on DNA synthesis (Figure 2). At 4 days post-balloon injury, both the AT_1 antagonist and the ACE inhibitor greatly reduced the number of SMCs which migrated from the media into the intima (Figure 3). Statistical analysis comparing the two treatment groups demonstrated that inhibition of SMC migration by treatment with DuP753 was significantly greater than that obtained by treatment with benazeprilat ($P < 0.05$).

Discussion

This study demonstrates that both the ACE inhibitor benazeprilat and the AT_1 antagonist DuP753 inhibited intimal lesion formation in Sprague Dawley rats after balloon injury. Although the ACE inhibitor significantly lowered blood pressure in normotensive Sprague Dawley rats, the AT_1 antagonist did not, thus demonstrating that compounds inhibiting the renin–angiotensin system can suppress vascular lesion formation via mechanisms other than blood pressure lowering. Furthermore, the finding that both an ACE inhibitor and an AT_1 antagonist are capable of inhibiting lesion development demonstrates the importance of Ang II in balloon–catheter-induced lesion formation.

The results using benazeprilat concur with the study by Powell et al.[16] in which lesions were inhibited to a similar extent by 3 mg/kg of the ACE inhibitor cilazapril. More potent lesion inhibition was shown to require doses higher than those necessary to obtain blood pressure lowering.[16] A recent preliminary report also described lesion inhibition with DuP753.[6] Previous studies, however, only examined compound effects on lesion size and did not determine whether lesion inhibition was due to inhibition of SMC migration or proliferation or both.

The AT_1 antagonist DuP753 was found to inhibit DNA synthesis in medial SMCs between 24 and 47 hours after

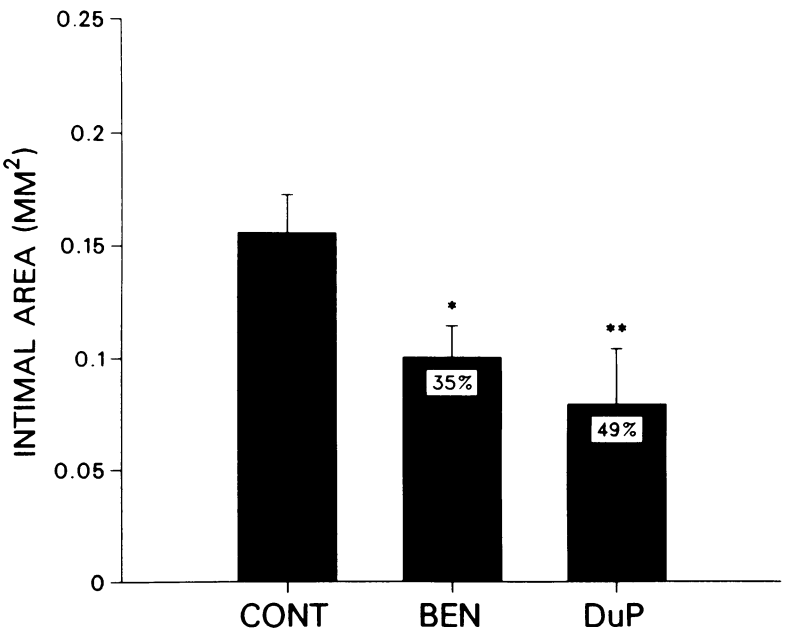

Figure 1. *Influence of the ACE inhibitor benazeprilat (BEN) and the AT_1 antagonist DuP753 (DuP) on intimal lesion size 12 days after balloon injury. *Benazeprilat (3 mg/kg/day) versus control (CONT) $P < 0.05$; **DuP753 (10 mg/kg/day) versus control $P < 0.01$. Values are mean ± SEM. Percentage within bars represents percent inhibition compared with control.*

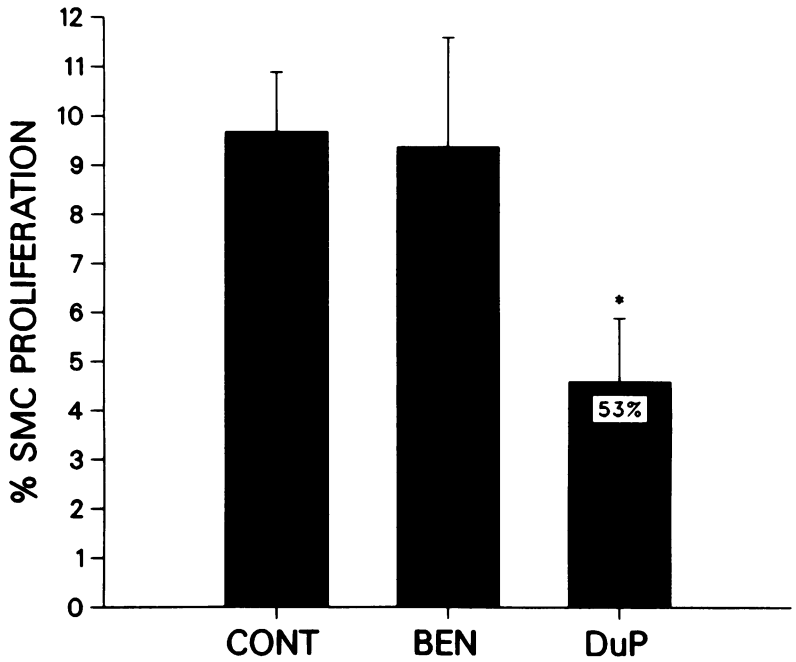

Figure 2. *Influence of the ACE inhibitor benazeprilat (BEN) and the AT_1 antagonist DuP753 (DuP) on % proliferation of medial smooth muscle cells (SMC) 24–47 hours after balloon injury. *DuP753 (10 mg/kg/day) versus control P < 0.05. Values are mean ± SEM. Percentage within bars represents percent inhibition compared with control.*

injury. This finding is in agreement with a recent study demonstrating that Ang II induces SMC proliferation *in vivo* in both the normal and balloon-injured vessel wall.[17] Our *in vivo* results correlate well with the *in vitro* study by Chiu et al.[18] demonstrating that DuP753 blocks Ang II-stimulated DNA synthesis in cultured SMC. There are several possibilities as to why the AT_1 antagonist inhibited SMC replication whereas the ACE inhibitor did not. One possibility is that treatment with the ACE inhibitor did not totally suppress vessel wall ACE levels. A recent study demonstrating significant levels of ACE in the injured vessel wall despite ACE inhibitor treatment[19] supports this hypothesis. If Ang II were produced locally in the vessel wall despite benazeprilat administration, it follows that treatment with DuP753, which potently blocks the SMC Ang II, AT_1 receptor,[4] would be a more effective way to inhibit SMC proliferation. Although 3 mg/kg/day of benazeprilat produces a maximal antihypertensive response,[12] a higher dose may result in a greater reduction in vessel wall ACE and thereby effect SMC proliferation.

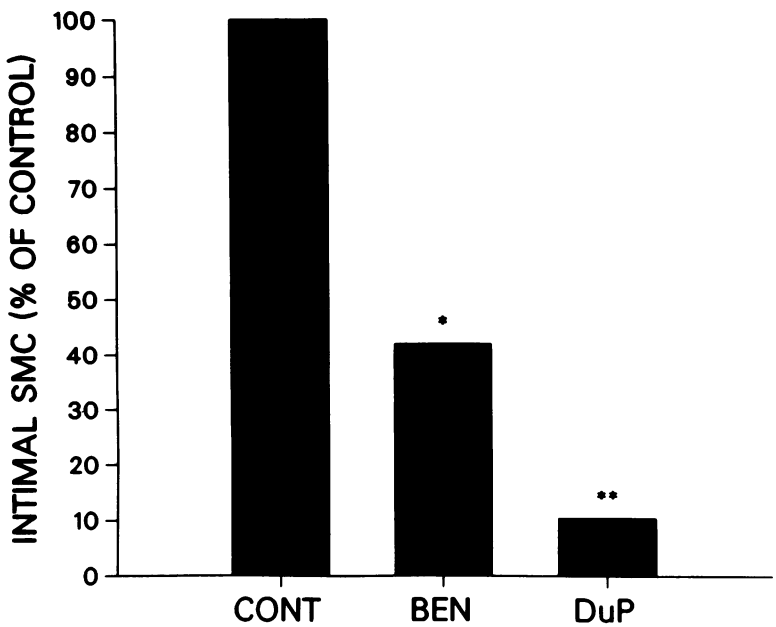

Figure 3. *Influence of the ACE inhibitor benazeprilat (BEN) and the AT_1 antagonist DuP753 (DuP) on the number of intimal smooth muscle cells (SMC) at 4 days after balloon injury. At this time point, the number of intimal SMC represents number of SMC that have migrated from the media into the intima. *Benazeprilat (3 mg/kg/day) versus control (CONT) P < 0.05; **DuP753 (10 mg/kg/day) versus control P < 0.001. DuP753 versus BEN P < 0.05. Values are means of two experiments (n = 4–5, each). Ranges: BEN = 38–52% of control; DuP753 = 7–20% of control.*

Benazeprilat, 10 mg/kg/day, was found to decrease DNA synthesis by 33%; however, the reduction did not achieve significance (Prescott and Reidy, preliminary data). Another possible reason why the AT_1 antagonist reduced SMC replication, whereas the ACE inhibitor did not, is that Ang II was produced locally via a non–ACE-dependent pathway. Two groups have demonstrated that Ang II can be produced in the vessel wall via an Ang I-degrading enzyme, which is distinct from ACE.[20,21]

Our study also demonstrates that balloon–injury-induced lesion formation can be reduced by inhibition of SMC migration in the absence of an effect on SMC replication. Benazprilat had no effect on SMC proliferation and yet demonstrated inhibition of intimal lesion size. At day 4, benazeprilat significantly decreased the number of intimal SMCs. Since in the balloon injury model, SMCs are first observed between days 3 and 4,[7–9] measurement of the number of intimal SMCs at day 4 was considered to represent the extent of SMC migration from the media. The present study agrees with previous studies using thrombocytopenic animals[10] or animals treated with anti-PDGF antibody[11] in which intimal lesions were reduced despite the fact that no effect on SMC replication was detected. These findings correlate well with a study demonstrating that PDGF is an important chemotactic agent but is not a mitogen *in vivo*.[22] Thus it is clear that migration is an important stage in the development of arterial lesions after balloon catheter-induced injury in rats. Our demonstration of inhibition of SMC migration with DuP753 correlate well with a study by Bell and Madri demonstrating a decrease in Ang II-stimulated SMC migration with administration of sar^1, ile^8-angiotensin II,[23] a potent antagonist of the vascular SMC AT_1 receptor.[4]

Although our results, as well as other recent studies,[10,11] demonstrate that balloon–catheter-induced lesion development can be prevented by inhibiting migration alone, the dissimilarity between the Baumgartner balloon catheter injury model in rats and transluminal angioplasty as performed in humans should be noted. When normal vessels are balloon injured using the Baumgartner technique, lesion formation is due to both migration of SMCs from the media to the intima and subsequent proliferation of SMCs within the intima.[7–9,15,24,25] In humans, however, transluminal angioplasty is performed in vessels already occluded by SMC-rich atherosclerotic plaque. Thus, in human vessels after angioplasty (as opposed to the rat balloon injury model) the importance of SMC migration in restenotic lesion formation is unknown. The fact that treatment with the ACE inhibitor cilazapril treatment did not reduce restenosis in the recently reported MERCATOR restenosis trial[26] suggests that inhibition of SMC migration alone may not be sufficient. Our study suggests that to inhibit restenosis after angioplasty, inhibition of both SMC migration and proliferation may be more beneficial than inhibition of SMC migration alone. Although in our study only the AT_1 antagonist inhibited both SMC events, an ACE inhibitor given at doses higher than those necessary to obtain blood pressure lowering may also inhibit both SMC migration and proliferation.

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