Trandolapril Inhibits Atherosclerosis in the Watanabe Heritable Hyperlipidemic Rabbit

Aram V. Chobanian, Christian C. Haudenschild, Cynthia Nickerson, and Susan Hope

The effects of trandolapril (0.25 mg/kg body wt per 48 hours) on aortic atherosclerosis were examined in the Watanabe heritable hyperlipidemic rabbit treated from 3 to 12 months of age. Trandolapril caused a significant decrease in atherosclerotic involvement of the intimal surface of total aorta from 56.3±5.0% in control Watanabe rabbits to 35.0±4.1% with treatment (p<0.01). The largest reductions were observed in descending thoracic aorta where 21.8±5.7% of intimal surface was involved in the trandolapril-treated animals versus 54.4±7.7% in the control group (p<0.01). Significant decreases also occurred in ascending aorta/arch and abdominal aortic segments. Cholesterol content of descending thoracic aorta was also significantly reduced in the trandolapril-treated rabbits. The atherosclerotic plaques in aorta from trandolapril-treated rabbits appeared to contain less foam cells and relatively greater amounts of connective tissue than those from control animals. These studies indicate that trandolapril inhibits aortic atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. The similarity in results between the current study and that using captopril suggests that the antiatherosclerotic action of trandolapril and captopril represents a class effect related to angiotensin converting enzyme inhibition. (Hypertension 1992;20:473–477)

KEY WORDS • trandolapril • angiotensin converting enzyme inhibitors • atherosclerosis • rabbit studies

Recent studies have indicated that angiotensin converting enzyme (ACE) inhibitors may alter the arterial response to injury. Powell et al reported that cilazapril and captopril reduced the degree of intimal plaque formation after balloon injury of normal rat carotid artery. Our laboratory demonstrated that captopril inhibits the development of aortic atherosclerosis in the Watanabe heritable hyperlipidemic (WHHL) rabbit, an animal model characterized by hyperlipoproteinemia secondary to a deficiency in the cellular receptor for low density lipoproteins. Aberg and Ferrer also reported that captopril inhibits atherosclerosis in arteries from the cholesterol-fed Macaca fascicularis monkey. It has not been determined as yet whether the antiatherosclerotic action of captopril is related to its inhibition of the ACE or to some other unrelated action of captopril.

The present study was undertaken to assess whether the drug trandolapril, which unlike captopril is a non-sulfhydryl-containing ACE inhibitor, has antiatherosclerotic effects in the WHHL rabbit. The protocol was essentially identical to that used in the captopril study to allow appropriate comparisons between the drugs.

**Methods**

Twenty WHHL rabbits (10 male and 10 female) were included in the study. The rabbits were weaned at 2 months of age and fed 120 g/day high fiber rabbit chow (Agway Prolab, Syracuse, N.Y.). The details of the breeding and feeding procedures are summarized in our prior publications.2-4

At 3 months of age, nine rabbits were randomly assigned to the control group and 11 were assigned to the trandolapril-treated (0.25 mg administered every other day in the food) group. The food was coated with 4% Karo syrup to facilitate adherence of the drug and to improve palatability. The Karo syrup was added to the diets of both control and trandolapril-treated rabbits. In keeping with the captopril study, the dose of trandolapril selected was the amount that lowered serum ACE activity by greater than 50% for at least 4 hours after ingestion. Because of the prolonged duration of action of trandolapril, which has also been observed in other studies, the drug was only given on alternate days with persistence of serum ACE inhibition at the end of the 48-hour dosing interval.

Systolic blood pressure and heart rate were measured monthly using a tail-cuff and photoelectric cell detector.6 Serum cholesterol was determined monthly after an overnight fast using an enzymatic technique as previously described.4

The rabbits were killed at 12 months of age with an intravenous injection of pentobarbital (100 mg/kg). After whole body perfusion with formalin, the aorta was removed, the adventitia was dissected away, and the tissue was immersion-fixed in formalin.4 The aorta was opened, and photographs were obtained as described.4
Representative segments of atherosclerotic plaque from descending thoracic aorta were removed for light microscopy, and microscopic sections were stained with hematoxylin and eosin. In addition, segments of grossly normal aortic wall were examined microscopically to exclude the presence of underlying disease in such areas.

The photographs of the inner surface of the aorta were projected onto the magnetic tablet of a manually operated image analyzer. The percentage involvement of ascending aorta and arch, descending thoracic aorta, and abdominal aorta was determined by two separate observers who were blinded regarding therapy. The individual regions of aorta were then separated, and their cholesterol content was measured as described.

Statistical analyses of data on blood pressure, heart rate, serum cholesterol, and body weight were performed by two-way analysis of variance with corrections made for repeated measures using the SAS program (Statistical Analysis System, SAS Institute Inc., Cary, N.C.). Aortic surface measurements were analyzed by Wilcoxon rank sum test. Aortic cholesterol values were compared using the Student's t test for independent samples.

**Results**

**Clinical Data**

No clinical adverse effects were observed in any of the control or trandolapril-treated WHHL rabbits throughout the period of study. Basal body weight and rate of weight gain were similar in both groups (Figure 1A). Serum cholesterol concentration at the initiation of the study averaged 779 mg/dl in the control animals and 751 mg/dl in the trandolapril group. No significant differences in serum cholesterol levels were present between the two groups at any of the subsequent time points (Figure 1B). Peak levels of serum cholesterol were observed in both groups at approximately 6 months of age with gradual decreases over the remaining period.

At the initiation of the study at 3 months of age, systolic blood pressure averaged 106±3.3 (mean±SEM) mm Hg in the control animals and 103±3.2 mm Hg in the trandolapril group (Figure 1C). From the first month of treatment onward, trandolapril caused a significant decrease in systolic blood pressure when compared against untreated rabbits, the differences generally averaging about 20–25 mm Hg. The average systolic blood pressures with treatment were in the 85–90 mm Hg range. None of the treated animals demonstrated clinical evidence of hypotension. Heart rate showed no consistent trend with treatment (Figure 1D).

**Aortic Surface Involvement**

Trandolapril caused a significant reduction in aortic atherosclerosis with decrease in total aortic surface involvement from 56.3±5.0% in the control group to 35.0±4.1% (p<0.01) with treatment (Table 1). The reductions appeared greatest in the descending thoracic
TABLE 2. Effects of Trandolapril on Aortic Cholesterol Content

<table>
<thead>
<tr>
<th>Aortic region</th>
<th>Control</th>
<th>Trandolapril</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(% intimal surface)</td>
<td>(% intimal surface)</td>
</tr>
<tr>
<td>Total aorta</td>
<td>56.3±5.0</td>
<td>35.0±4.1*</td>
</tr>
<tr>
<td>Ascending and arch</td>
<td>95.0±1.9</td>
<td>82.0±3.2*</td>
</tr>
<tr>
<td>Descending thoracic</td>
<td>54.4±7.7</td>
<td>21.8±5.7*</td>
</tr>
<tr>
<td>Abdominal</td>
<td>39.0±4.0</td>
<td>24.5±3.7†</td>
</tr>
</tbody>
</table>

Data represent the mean±SEM. *p<0.01; †p=0.02.

aorta (54.4±7.7% in control WHHL rabbits as compared with 21.8±5.7% in treated animals, p<0.01). However, significant reductions were also observed in the ascending aorta/arch (p<0.01) and in the abdominal aorta (p=0.02) segments.

Aortic Cholesterol Content

Total aortic cholesterol content (Table 2) was significantly lower in the descending thoracic aorta of trandolapril-treated animals (16.0±3.4 mg/g wet wt) as compared with the control group (29.3±3.5 mg/g wet wt) (p<0.01). No statistically significant differences were observed in the ascending aorta/arch and abdominal aorta, although the average cholesterol content in the abdominal aorta of treated rabbits (16.3±3.7 mg/g wet wt) was approximately 30% less than in the control group (22.0±3.3 mg/g wet wt).

Microscopic Examination

The typical lesions present in the descending thoracic aorta of the untreated WHHL rabbits showed intimal thickening covered by endothelium. The intima contained many foam cells interspersed with areas of necrosis and accumulated extracellular matrix. In contrast, even the most extensive lesions in the trandolapril-treated animals showed relatively few foam cells and relatively large amounts of extracellular matrix, often associated with areas of calcification. The microscopic sections taken from areas of aorta that appeared normal on gross inspection failed to reveal evidence of underlying disease in these regions. Representative lesions from control and trandolapril-treated WHHL rabbits are shown in Figure 2.

Discussion

The present studies indicate that the ACE inhibitor trandolapril inhibits aortic atherosclerosis in the WHHL rabbit. The degree of inhibition of atherosclerosis by trandolapril in the current study and of captopril in a prior investigation was broadly comparable; however, the inhibition by trandolapril, in terms of area involvement, was significant in all regions of aorta, whereas the captopril effect was evident primarily in the descending thoracic aorta. These modest differences may have been due to animal variability, although other drug effects such as duration of action or variations in tissue affinity of the drugs cannot be ruled out. Trandolapril, with its very long duration of action, probably provided more sustained inhibition of serum ACE activity than did captopril, but it is unknown whether the drugs differed in their ability to inhibit vascular ACE. The dose of both drugs was considerably greater than that used clinically in humans, although the dose we selected was designed to inhibit serum ACE activity to a similar extent as that typically observed in humans. Furthermore, previous studies have demonstrated species differences in drug kinetics with higher amounts of ACE inhibitors required in rabbits than in humans to inhibit angiotensin II production. The mechanisms for the antiatherosclerotic action of trandolapril and captopril have not been delineated, but the current data strongly suggest that a class effect related to ACE inhibition is involved. The findings minimize any likelihood that the sulfhydryl moiety of captopril plays a role. The blood pressure-lowering effect of the drugs remains of interest with respect to their antiatherosclerotic action even though the reductions were within a generally accepted normal range of blood pressure. Epidemiological studies have demonstrated low rates of cardiovascular complications in human subjects with the lowest levels of blood pressure. In addition, several antihypertensive drugs including β-adrenergic blockers and calcium channel blockers have been shown to inhibit atherosclerosis in cholesterol-fed animals, although the relation of such effects to blood pressure lowering or other hemodynamic actions has not been delineated. Future studies in which nondepressor doses of ACE inhibitors are examined will be of interest to help clarify this issue.

Autocrine or paracrine effects of angiotensin II could be important in cardiovascular regulation and in the response to vascular injury. Individual components of the renin-angiotensin system and generation of angiotensin II have been demonstrated in vascular cells. Cell culture studies have shown that angiotensin II can stimulate cell growth and induce either hypertrophy or hyperplasia, depending on the culture conditions, the presence of other growth factors, or the state of activation of the cells. In serum-free medium, angiotensin II may induce hypertrophy of rat aortic smooth muscle cells (SMC) and increase expression of protooncogenes and of the A-chain of platelet-derived growth factor. Angiotensin II also can cause hyperplasia of human aortic SMC and 3T3 fibroblasts in the presence of serum. Vascular cells that are responding to injurious stimuli and are thereby phenotypically altered may be particularly sensitive to the effects of angiotensin II. Tissue absences of serum in the culture medium, angiotensin II has been reported to stimulate proliferation in cultured aortic SMC from spontaneously hypertensive rats but not in SMC from Wistar-Kyoto normotensive controls. In vivo studies in which angiotensin II was administered chronically with osmotic minipumps have demonstrated increased thymidine incorporation into SMC of rat aorta and carotic artery. The stimulation of thymidine incorporation was greatest in the arterial intima and was magnified after balloon catheter injury. Studies from our own laboratory have shown that in vivo administration of...
angiotensin in the rat causes modulation of the steady-state messenger RNA levels of connective tissue proteins as fibronectin, collagen, and tropoelastin.19 Such effects could influence the remodeling process occurring in response to arterial injury.

Increased proliferation of arterial SMC and their accumulation in the intima are important features in the development of atherosclerosis, and inhibition of such proliferation might prove effective in reducing plaque formation. Atherosclerotic lesions are composed of a mixed cell population that includes macrophages and lymphocytes as well as SMC and endothelial cells, and ACE inhibitors potentially could act on any of these cell types. In addition, recent studies have shown that inhibition of the renin-angiotensin system with either the ACE inhibitor lisinopril or the angiotensin II antagonist [sar⁴, ile⁹]angiotensin II, increases the migration rate of cultured bovine aortic endothelial cells and the activity of urokinase-like plasminogen activator.20 Such effects, if present in vivo, could be beneficial by accelerating the rate of repair of damaged intima and by reducing its thrombogenicity. These studies also have shown that lisinopril reduces SMC migration, another potentially important antiatherosclerotic action.

The cellularity of aortic plaques in our WHHL rabbits appeared less in both trandolapril- and captopril-treated animals; both macrophage and SMC populations appeared to be affected. However, although it is tempting to speculate that such changes occurred because of a growth inhibitory action of these drugs, it should be remembered that any therapeutic intervention that causes prevention or regression of atherosclerosis would lead to reduced cellularity of the intima.

The pronounced antiatherosclerotic effects of ACE inhibition in the WHHL rabbit are similar to those reported in cholesterol-fed cynomolgus monkeys treated with captopril.3 In contrast, despite their favorable action in cholesterol-fed rabbits,9,11,21 neither

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**Figure 2.** Panel A: Photomicrograph shows cross section through a representative intimal lesion in descending thoracic aorta of a 12-month-old control Watanabe heritable hyperlipidemic (WHHL) rabbit. Note the high cellularity of the plaque and the presence of foam cells and cholesterol crystals. Panel B: Photomicrograph shows representative intimal lesion in descending thoracic aorta of a 12-month-old trandolapril-treated WHHL rabbit. This intimal plaque is smaller, shows less cell density, and appears to have more extracellular matrix than the lesion in Panel A. Both panels, hematoxylin-eosin; original magnification ×60.
\(\beta\)-blockers nor calcium channel blockers have inhibited atherogenesis in the WHHL rabbit,\(^{22-24}\) and nifedipine has proven to be ineffective in the cholesterol-fed cynomolgus monkey.\(^{25}\) Thus, differences between the mechanisms for the antiatherosclerotic effects of ACE inhibitors and other antihypertensive drugs would seem likely.

Several studies have been performed lately on the effects of ACE inhibitors on plaque development after balloon injury or angioplasty. Cilazapril and captopril have both been reported to reduce myointimal thickening in rat carotid artery after balloon injury.\(^{1}\) The effect appears dose-related and may be potentiated by concurrent therapy with heparin.\(^{26}\) On the other hand, verapamil has been reported to have no effect in the same model and hydralazine may cause much less plaque inhibition than the ACE inhibitors, despite similar lowering of blood pressures.\(^{26}\) The high rate of SMC proliferation in the neointima after balloon injury\(^{27}\) appears associated with high rates of production of platelet-derived growth factor A-chain\(^{28}\) and transforming growth factor \(\beta-1.\)\(^{29}\) ACE inhibitors appear to reduce the proliferative response in this model. In addition, although ACE inhibitors may inhibit restenosis in rat carotid artery, the effect may not be present in other models of vascular injury. For example, studies in baboons showed no effect of cilazapril on plaque formation after balloon catheter injury of superficial femoral artery or after carotid endarterectomy.\(^{30}\) Furthermore, a recent randomized, placebo-controlled clinical trial in patients treated by percutaneous transluminal coronary angioplasty failed to demonstrate a significant effect of cilazapril on either restenosis, as assessed by coronary angiography, or on clinical outcome.\(^{31}\) No adequate explanation has been provided for these seemingly disparate findings, although differences in species, types of vascular injury used, or treatment protocols all need to be considered.

The current findings, though of considerable interest, should not be generalized to clinical practice. Clinical trials are required to determine whether ACE inhibitors will reduce atherosclerosis and its complications in humans.

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