Eplerenone Suppresses Constrictive Remodeling and Collagen Accumulation After Angioplasty in Porcine Coronary Arteries

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Background—Coronary artery angioplasty triggers healing that causes constrictive remodeling. Because collagen accumulation correlates with constrictive remodeling and aldosterone has been implicated in collagen accumulation, we examined how aldosterone and the mineralocorticoid receptor antagonists spironolactone and eplerenone affect remodeling and collagen in porcine coronary and iliac arteries after angioplasty.

Methods and Results—Twenty-four pigs were allocated into 4 treatment groups: oral eplerenone (100 mg/d), oral spironolactone (200 mg/d), subcutaneous aldosterone (400 mg/d), or no treatment. Twenty-eight days after angioplasty of the coronary arteries, eplerenone increased total vessel area by 30% (P < 0.05) and luminal area by nearly 60% (P < 0.05) compared with the no-treatment group, without affecting neointima size. These effects were accompanied by a 65% reduction in neointimal and medial collagen density (both P < 0.05). Spironolactone was less effective, and aldosterone tended to exert opposite effects on coronary artery structure after angioplasty. These effects were not observed in angioplastied iliac arteries.

Conclusions—Eplerenone attenuates constrictive remodeling after coronary artery angioplasty by mechanisms involving reduction in collagen accumulation, which thus appears to be an important contributor to constrictive remodeling of angioplastied coronary arteries. (Circulation. 2001;104:467-472.)

Key Words: angioplasty ■ arteries ■ remodeling ■ aldosterone ■ collagen

Constrictive remodeling is a major contributor to restenosis after coronary artery angioplasty,1,2 becoming apparent 1 to 6 months after angioplasty2 and resulting in luminal narrowing.1 Studies after angioplasty of porcine coronary arteries have confirmed the importance of remodeling, demonstrating that the late luminal loss substantially exceeds that which can be attributed to neointima formation.3 This process can be ameliorated by endoluminal stents, which may stimulate neointima overgrowth and subsequent restenosis.4

Despite the importance of constrictive remodeling in restenosis, it is poorly understood. Collagen accumulation is one potential factor contributing to restenosis and remodeling after angioplasty.5 Extracellular matrix density increases in balloon catheter–injured arteries,6 and in angioplastied coronary arteries it is highest in vessels in which constrictive remodeling is present.7 Because aldosterone increases collagen, leading to aortic fibrosis,7 we examined, in angioplastied porcine coronary arteries, whether chronic administration of the mineralocorticoid antagonist eplerenone affects collagen accumulation, constrictive remodeling, and the size of the developing neointima. Eplerenone is an aldosterone antagonist in which the 17α-thioacetyl group of spironolactone has been replaced with a carboxy group, conferring much higher selectivity for mineralocorticoid receptors than is the case for spironolactone, and has been shown to be active against both the epithelial and nonepithelial effects of aldosterone.8,9 We demonstrate that eplerenone attenuates constrictive remodeling after coronary artery angioplasty and reduces collagen accumulation. Spironolactone treatment was less effective. These findings were specific for angioplastied coronary arteries and demonstrate the importance of collagen accumulation in constrictive remodeling.

Methods

Animals, Surgical Procedures, and Drug Treatments

Male Boston minipigs (26 to 40 weeks old, 40 to 60 kg) were from Monash University, Clayton, Australia. Angioplasty was performed on up to 2 coronary arteries and 2 comparably sized deep circumflex branches of the external iliac artery in each pig. Two of the 3 main coronary branches (the right, the left anterior descending, and the left circumflex coronary arteries) were dilated in a random manner in each pig, except when difficulties were encountered because of poor catheter engagement or arrhythmias.
All animals received aspirin 300 mg/d PO, commencing 7 days before surgery and continuing throughout the study. Verapamil (120 mg PO, Knoll) was administered 12 hours before surgery. The pigs were premedicated with acepromazine (0.1 mg/kg IM, Delta) and atropine sulfate (1.2 mg IM, Delta). Anesthesia was induced with propofol (150 to 200 mg IV, ICI) and maintained with isoflurane (Abbott).

Angioplasty was performed with an 8F JL4 guiding catheter through a sheath inserted into the right common carotid artery after intravenous heparin (15 000 U, Fisons). Angiography was performed after intra-arterial glycyr trinitrate (200 μg, Fisons) with loxaglate (Hexabrix, Mallinckrodt), recording in the left anterior oblique view (25°) for the coronary arteries and in the straight anteroposterior view for the iliac vessels. The arteries were dilated with human angioplasty balloon catheters (semicompliant, 20 mm long), which were oversized according to the manufacturer-specified balloon size with a balloon-to-artery ratio of 1.3:1 to 1.5:1. The balloon catheter was inflated to 10 atm for 30 seconds with 3 separate inflations separated by 1 minute in the coronary and iliac vessels. To more easily identify injured segments of vessels, the most proximal segment of each artery was injured. Angiography was repeated 28 days later to confirm vessel patency.

Angioplasty was performed in 4 groups, each with 6 animals: a control (untreated) group, a group receiving eplerenone (Searle) 100 mg/d PO, a group receiving spironolactone (Searle) 200 mg/d in 2 divided oral doses, and a group receiving aldosterone (Sigma) 400 μg/d by continuous subcutaneous infusion. Treatment with eplerenone and spironolactone was commenced 7 days before angioplasty and then continued for 28 days. Aldosterone infusion commenced immediately after angioplasty with subcutaneous osmotic minipumps and continued for 28 days. No differences were seen in measured food intake throughout the study between groups. The study was approved by the Baker Institute’s Experimentation Ethics Committee.

Vessel Isolation and Processing for Histology

Animals were anesthetized, heparinized, and killed with ketamine and pentobarbitone 28 days after angioplasty. The aorta, heart, and iliac vessels were exposed, and the coronary and circumflex iliac arteries were perfused with 4% formalin in PBS, pH 7.4, at 100 to 150 mm Hg, with the aorta cross-clamped and the right and left atrial appendages incised on infusion via a cannula. To isolate the deep circumflex iliac arteries, the distal aorta, internal iliac arteries, and external iliac arteries distal to the deep circumflex branch were ligated, and a cannula was introduced into the distal aorta for infusion of formalin, which was drained via the inferior vena cava. Vessels were stored in 4% formalin and then cross-sectioned every 3 mm, dehydrated in ethanol and xylene, embedded serially in paraffin, and then sectioned (4 μm) and stained with hematoxylin-eosin and Masson’s trichrome stain with orcein or picrosirius red.

Vessel Injury Assessment

All segments of coronary and circumflex iliac arteries were serially examined, and the site of the most severe injury was identified. These regions were used for all histological measurements. The severity of injury to the arteries was classified according to the angle of injury by determination of that part of the vessel’s circumference at which the tunica media was abraded and the adventitia exposed. The sides of this gap angle were drawn from the center of the lumen, tracing the respective vessel regions, and then calculating areas by planimetry. The adventitia is defined as the area between the EEL and periadventitial tissues; overall vessel size was defined as the area circumscribed by the EEL. The media is defined as the region between the EEL and the IEL; when the IEL was missing, it was defined as areas of remnants of medial tissue (ie, well-organized smooth muscle cells [SMCs] with intervening elastic fibers). The neointima comprises the region between the lumen and the IEL and, when the elastic lamina was missing, the area between the lumen and remnants of medial tissue or the EEL. The lumen area is defined as the region circumscribed by the intima/neointima-lumen border.

Collagen in coronary arteries stains green with Masson’s trichrome stain, and elastin stains dark red with orcein. The contribution made by collagen and elastin to different artery regions was quantified by use of a computer-interfaced color imaging system (Optimus Bioscan 2, Thomas Optical Measurement System, Inc) to measure the fractional areas of green and dark red in stained sections. To avoid color balance variation, staining of all sections was performed at the same time on 1 day. We sampled 8 to 10 pixels of color that defined the collagen (green) and elastin (dark red) staining; once a standard for the particular slice/section was set, by picking individual pixels to represent a color, it was used to measure all the sections from the different groups of animals. This threshold did not require adjustment, and measurements were made over a 4-day period. Collagen and elastin measurements were performed on the entire media, neointima, or adventitia. Up to 5 consecutive sections were measured from each vessel, with coefficients of variation for the morphometric and collagen measurements being 3.4% and 4.9%, respectively. Values for collagen content in vessels by this procedure were not different from those obtained with the picrosirius red procedure; in the neointima of serial sections of 7 arteries from control pigs, they averaged 19.1 ± 1.60% and 20.7 ± 1.5% with trichrome and picrosirius red staining, respectively (P > 0.20).

Statistical Analysis

Data are presented as mean ± SEM of the number of arteries examined. Differences between groups were assessed by 1-way ANOVA after testing for normality by the Kolmogorov-Smirnov test; post hoc analyses used the Newman-Keuls test. Data that failed the test for “normality” were analyzed by nonparametric ANOVA, with differences analyzed by the Mann-Whitney rank sum test. Linear regression analyses and paired t tests were performed by Sigmastat.

Results

Injury by Angioplasty in Coronary and Iliac Arteries

Eplerenone, spironolactone, and aldosterone treatments were well tolerated, as indicated by general well-being and normal food intake.

Twenty-eight days after angioplasty, the coronary arteries exhibited disrupted IEL, laceration of the media, and exposure of the IEL (Figure 1, top). The severity of injury to coronary arteries, measured by gap angle, was similar (P > 0.05) in the groups, averaging 114° (Table 1). Injury assessed by whether the IEL, EEL, and media were fractured/lacerated also indicated no differences between groups (P > 0.05; Table 1), as did measurements of IEL fracture length to IEL circumference (not shown). Injury levels in the circumflex iliac arteries were also similar in the 4 groups. In these vessels, there was no evidence for any laceration of the media and/or exposure of the EEL (Figure 1, bottom), despite similar levels of oversizing (manufacturer’s balloon size specifications/vessel diameter before injury, 1.449 versus...
Eplerenone and Remodeling of Coronary Arteries

Because the relationship between vessel cross-sectional area (VA) and gap angle (GA) (VA = 3.90 – 0.00288GA over the gap angle range 20° to 240°) was weak (R² = 0.03; SE of constant = 0.2, SE of slope = 0.002; n = 87 sections from untreated angioplastied coronary arteries), no corrections were made to indices of vessel remodeling for the small, nonsignificant differences in GAs.

The overall size of angioplastied coronary arteries increased by ∼30% in animals treated with eplerenone compared with untreated vessels (Figure 2; P < 0.05). Vessel luminal cross-sectional areas in the eplerenone-treated animals was even more markedly increased, by ∼60% over control (P < 0.05). Although neointima size in eplerenone-treated animals appeared to be lower than that of controls, the difference was not significant (P > 0.10); consequently, eplerenone markedly reduced the calculated neointimal area/vessel area ratio (P < 0.05 from control; Figure 2). These results indicate that eplerenone attenuates constrictive remodeling of angioplastied coronary arteries. The pattern of effects after spironolactone treatment was qualitatively similar to that of eplerenone, although the effects were smaller and not statistically significant (Figure 2; P > 0.05). Because higher doses of spironolactone (eg, 200 mg twice daily) are poorly tolerated by the pigs (D.R., unpublished observations), we did not investigate whether such doses might attenuate constrictive remodeling to the extent seen with eplerenone.

To determine whether these effects of eplerenone on remodeling reflected aldosterone antagonist activity, we also determined the extent to which elevating circulating aldosterone levels affected the constrictive remodeling. Aldosterone did not reduce vessel cross-sectional area or lumen size compared with control coronary arteries (P > 0.10, Figure 2) or increase neointimal cross-sectional area or the intima area/vessel area ratio (P > 0.05, Figure 2), probably reflecting significant local production.14

Aldosterone Antagonists and Angioplastied Iliac Arteries

Eplerenone did not affect total cross-sectional area, lumen area, or neointimal area in the injured circumflex iliac arteries.
after angioplasty ($P>0.05$ versus control; Table 2). Spironolactone was without significant effect ($P>0.05$). Aldosterone, however, did increase neointima area ($P<0.05$ versus control; Table 2).

**Eplerenone and Collagen in Angioplastied Coronary Arteries**

Because constrictive remodeling of angioplastied coronary arteries has been associated with increases in collagen, we investigated whether eplerenone might attenuate constrictive remodeling by reduction in collagen accumulation. Eplerenone reduced collagen content in the neointima and media of angioplastied coronary arteries (Figures 3 and 4), by 65% in the neointima and by a similar amount in the media, compared with untreated arteries ($P<0.05$; Figures 3 and 4). Neoadventitial collagen was unaffected (Figure 4; $P>0.05$). Spironolactone therapy did not affect collagen in these vessels (Figure 4), whereas aldosterone increased medial collagen density by $\sim 75\%$ ($P<0.05$ versus control; Figure 4), but not neointimal or neoadventitial collagen ($P>0.05$ versus control).

**Aldosterone Antagonists and Elastin Content in Coronary Arteries**

Elastin density was highest in the media of the angioplastied coronary arteries and lowest in the neoadventitia (Figure 4) and was unaffected by eplerenone, spironolactone, or aldosterone.

### TABLE 2. Parameters of Circumflex Iliac Artery Structure 28 Days After Angioplasty

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>No. of Vessels</th>
<th>Vessel Area, $\times10^6$ $\mu m^2$</th>
<th>Lumen Area, $\times10^6$ $\mu m^2$</th>
<th>Intimal Area, $\times10^6$ $\mu m^2$</th>
<th>IA/VA Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>$1.52\pm0.27$</td>
<td>$0.77\pm0.24$</td>
<td>$0.13\pm0.02$</td>
<td>$0.096\pm0.021$</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>10</td>
<td>$1.43\pm0.14$</td>
<td>$0.64\pm0.14$</td>
<td>$0.12\pm0.02$</td>
<td>$0.089\pm0.013$</td>
</tr>
<tr>
<td>Eplerenone</td>
<td>7</td>
<td>$1.69\pm0.14$</td>
<td>$0.71\pm0.15$</td>
<td>$0.15\pm0.01$</td>
<td>$0.090\pm0.010$</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>9</td>
<td>$1.63\pm0.13$</td>
<td>$0.53\pm0.07$</td>
<td>$0.24\pm0.05^*$</td>
<td>$0.144\pm0.025$</td>
</tr>
</tbody>
</table>

IA and VA represent intima area and vessel cross-sectional area. Results are mean $\pm$ SEM. $^*P<0.05$ vs control (untreated animals).
Constrictive remodeling is a major mechanism responsible for restenosis after angioplasty. The mechanisms proposed for the constrictive remodeling include decreases in blood flow, generation of reactive oxygen species, and collagen accumulation. We provide evidence to support the hypothesis that collagen accumulation during healing after coronary artery angioplasty contributes to constrictive remodeling and restenosis. Our results indicate that mineralocorticoid antagonists such as eplerenone may attenuate the extent and/or frequency of restenosis by reducing collagen accumulation within media and neointima.

Although proliferation occurs early after coronary artery angioplasty, inhibitors of SMC proliferation are generally unsuccessful in preventing restenosis. This may reflect the low levels of cell proliferation in human restenotic lesions, so that approaches that attenuate remodeling may prove to be more effective. Our study demonstrates that eplerenone inhibits constrictive remodeling in porcine angioplastied coronary arteries; spironolactone, at double the dose, was without significant effect. Higher doses of spironolactone administered to rabbits, however, attenuate neointima formation in balloon catheter–injured iliac arteries and aorta. On Kagawa bioassay in adrenalectomized rats, eplerenone and spironolactone have equivalent potencies, and in human dose-ranging studies, eplerenone is 70% as active as spironolactone in reducing blood pressure, suggesting that higher doses of spironolactone might not add appreciably to its effectiveness. Aldosterone has been reported to increase neointima size in balloon catheter–injured iliac arteries but not aorta; in our studies, aldosterone differentially affected coronary and circumflex iliac arteries, increasing neointima size in iliac arteries only. The differential response of the coronary and circumflex iliac arteries to eplerenone and aldosterone may reflect the lower level of disruption in the circumflex iliac and a milder healing response.

Mineralocorticoids have been implicated in collagen accumulation in vessels. Because increases in collagen accumulation have been associated with constrictive remodeling of angioplastied arteries, we examined the extent to which mineralocorticoid antagonists affected collagen accumulation in coronary arteries. Eplerenone was much more efficacious in attenuating collagen accumulation than spironolactone, directly implicating collagen accumulation in the constrictive remodeling of angioplastied coronary arteries. The precise mechanism by which eplerenone attenuates collagen accumulation may involve actions via 1 enzyme systems involved in collagen biosynthesis/degradation during healing, perhaps by modulating tissue plasminogen activator-I. The resultant reduction in collagen density and collagen type composition in the media and neointima may prevent constrictive remodeling by reducing SMC and fibroblast migration. Type VIII collagen promotes SMC chemotaxis and matrix metalloproteinase synthesis. Such effects could alter the extent to which SMCs and fibroblasts initially populate the neointima. In addition, studies in pressure-distended arteries suggest that remodeling may involve a specific pattern of temporal and regional increases in the expression of collagen subtypes in vessels, also regulating the extent to which different cell types remodel extracellular collagen through cell-matrix interactions.

Study Limitations
A major limitation of this and most other restenosis models is the lack of a preexisting atherosclerotic lesion. As in human restenotic lesions, however, constrictive remodeling is the major mechanism contributing to luminal narrowing in porcine angioplastied coronary arteries. Also, porcine models of coronary artery restenosis appear to predict clinical outcomes with new therapies better than either rat or rabbit models. Although we demonstrate that therapy with eplerenone can be of benefit in the prevention of coronary artery restenosis, the pharmacological aspect of the study has several limitations. We did not examine the relationship between dose and the ability of eplerenone to inhibit constrictive remodeling, and the lack of any pharmacokinetic data for eplerenone in the pig prevented us from designing an optimal dose regimen. Similarly, the lack of pharmacokinetic and metabolic data in pigs for spironolactone limited our ability to elucidate why eplerenone was more efficacious than spironolactone in preventing constrictive remodeling. In humans, spironolactone is rapidly metabolized to a number of metabolites, including canrenone, 6-β-hydroxy-7α-thiomethylspironolactone, and 7α-thiomethylspironolactone; the latter has been implicated in adrenal cytochrome P450 destruction. It is possible that thiol metabolites are responsible for the inability of pigs to tolerate higher doses of spironolactone, preventing our attempt to achieve bioequivalence with eplerenone on constrictive remodeling of the coronary arteries.

Conclusions
Orally administered eplerenone is effective in attenuating collagen accumulation and constrictive remodeling of angioplastied porcine coronary arteries. Further studies into the
aldosterone-dependent mechanisms operating after angioplasty may provide novel insights as to how coronary artery restenosis can be prevented.

References
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_Circulation_. 2001;104:467-472
doi: 10.1161/hc3001.091458
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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