Selective ET\textsubscript{A} Receptor Antagonism Reduces Neointimal Hyperplasia in a Porcine Coronary Stent Model

Charles J. McKenna, MB, MRCP; Sandra E. Burke, PhD; Terry J. Opgenorth, PhD; Robert J. Padley, PhD; LaDonna J. Camrud, RN; Allan R. Camrud, RN; Jodi Johnson; Paula J. Carlson, BS; Amir Lerman, MD; David R. Holmes, Jr, MD; Robert S. Schwartz, MD

Background—As endothelin binds to ET\textsubscript{A} receptors, it stimulates vascular smooth muscle cell proliferation and may thus be pivotally involved in the pathogenesis of restenosis. This study assessed the ability of a potent and selective ET\textsubscript{A} antagonist to reduce neointimal hyperplasia in a porcine coronary artery stented injury model.

Methods and Results—Fifty-five pigs were randomized to receive placebo or the oral ET\textsubscript{A}-selective antagonist ABT147627 twice daily for 28 days in one of three doses: 0.75 mg/kg (low), 3.75 mg/kg (mid), and 10.0 mg/kg (high). Each underwent oversized stent deployment in two randomly assigned major epicardial coronary arteries. Three animals (5.5%) died as a consequence of stent thrombosis within 24 hours of the procedure. The remaining 52 animals (13 pigs per group) survived without complication until predetermined euthanasia at 28 days. In the placebo group, mean injury score was 1.73±0.80, with a mean neointimal response of 0.45±0.24 mm. By comparison, the low-dose group had a similar mean injury score of 1.79±0.75 with reduced neointimal response, 0.36±0.22 mm (P<0.01). Mean injury score in the mid-dose animals was significantly greater than in the placebo group (1.94±0.92; P<0.05). The neointimal hyperplasia associated with this injury was less than with placebo, although the difference did not reach statistical significance (0.40±0.25 mm; P=0.05). In the high-dose pigs, mean injury score was also significantly greater than in the placebo arm (1.93±0.73; P<0.05). Despite this, neointimal response was also significantly less (0.37±0.37 mm; P<0.01).

Conclusions—Oral, selective ET\textsubscript{A} receptor antagonism significantly reduced neointimal hyperplasia forming over porcine coronary stented injuries in the first 28 days. This strategy may have clinical potential for the limitation and treatment of coronary restenosis after percutaneous revascularization. (Circulation. 1998;97:2551-2556.)

Key Words: endothelin • angioplasty • restenosis

The endothelins (ETs) are a family of isopeptides (ET-1, ET-2, and ET-3) first isolated from porcine aortic endothelial cells in 1988. They are secreted predominantly by endothelial cells and after cleavage form the prepropeptide big ET (39 amino acids). Further cleavage by ET-converting enzyme results in the formation of the acidic 21-amino-acid ET polypeptides. All three peptides are encoded by different genes in human, porcine, and rat DNA and as such are structurally and pharmacologically distinct. Two receptors for ET, ET\textsubscript{A} and ET\textsubscript{B}, have been characterized by isolation and gene coding. ET\textsubscript{A} receptors predominate in the heart and vascular smooth muscle, whereas ET\textsubscript{B} receptors are found in endothelial cells, kidney, and central nervous tissue. The binding of ET to its receptor results in release of calcium from the sarcoplasmic reticulum and enhanced entry of extracellular calcium, resulting in an increase in intracellular free calcium. ET-1 is a potent vasoconstrictor in mammals and, in addition to its long-lasting pressor actions, it also induces mitogenesis in endothelial cells and vascular smooth muscle cells. Coronary vasoconstriction by ET-1 in dogs is mediated predominantly by ET\textsubscript{A} receptors. ET\textsubscript{A} receptors have a 10-fold higher binding affinity for ET-1 than ET-2, and the mitogenic effects of ET-1 can be prevented by selective ET\textsubscript{A} receptor antagonism. Both circulating and tissue ET immunoreactivity are increased in patients with advanced atherosclerosis, and tissue reactivity is associated with vascular smooth muscle and endothelial cells.

An increase in ET and big ET is observed in fully developed atherosclerotic plaques in humans, with ET\textsubscript{A} receptors predominating in the media of normal and diseased arteries. This evidence suggests that ET, via binding to ET\textsubscript{A} receptors, stimulates vascular smooth muscle cell proliferation and may also be pivotally involved in the pathogenesis of atherosclerosis. ET-1 immunoreactivity is also increased in patients after coronary angioplasty, both in the coronary sinus and in the distal segment of the injured artery. The level of reactivity correlates with the degree of mechanical stress applied to the arterial lesion. The infusion of ET-1 in
the rat carotid balloon injury model has been shown to worsen neointimal hyperplasia after mechanical injury.\textsuperscript{15,16} The neointimal hyperplasia observed in this model has been significantly reduced by nonselective and selective ET\textsubscript{A} receptor antagonism.\textsuperscript{18–19}

The present study was thus designed to assess the ability of the selective ET\textsubscript{A} antagonist ABT147627 to reduce neointimal hyperplasia in a porcine coronary stent injury model. Efficacy of this agent in this model would indicate its clinical potential for the limitation and/or treatment of coronary restenosis after percutaneous revascularization with stents.

Methods

Animals
This study was performed with the approval of the Animal Care and Use Committee of the Mayo Foundation. The juvenile domestic, crossbred porcine coronary injury model used has been described previously.\textsuperscript{3} Three days before the procedure, pigs were started on oral aspirin (325 mg), which was continued for the remainder of their course. General anesthesia was achieved with ketamine (3 mg/kg IM) and xylazine (30 mg/kg IM). Additional medication at the time of induction included atropine (1 mg IM) and antibiotic (flocillin, 1 g IM). During the stenting procedure, an intra-arterial bolus of heparin (10 000 U) was administered. Arterial access was obtained by cutdown of the right external carotid artery and placement of an 8F sheath. After the procedure, the wounds were closed and the pigs were returned to quarters on a normal diet.

Stent Placement
Two coronary arteries per pig were randomly assigned for deployment of tantalum wire-coil stents. Stents were 15 mm long, hand-crimped on 20-mm balloons, and delivered by standard angioplasty guide catheters and wires. The stents were inflated to 1.2 to 1.4 times the size of the reference vessel (based on arterial and nominal balloon sizes) to create significant arterial injury and thus ensure a measurable neointimal response.

Drug Treatment
The four drug treatment groups consisted of a placebo arm and three escalating doses of the ET\textsubscript{A} antagonist ABT147627. Oral drug was administered twice daily in capsule form at doses of 0.75 mg/kg (low), 3.75 mg/kg (mid), and 10.0 mg/kg (high). The placebo group received capsules containing cornstarch. Therapy was started 1 hour before stenting and continued for the 28 days until euthanasia.

Before euthanasia, the animals underwent a pressor challenge using big ET-1 to determine the adequacy of ET antagonism. The pigs were anesthetized identically to the above regimen. Arterial blood pressure was continuously monitored with a fluid-filled transducer connected to a carotid artery sheath. Big ET-1 (0.3 nmol/kg) was injected when the animal was hemodynamically stable at time 0, and systolic, diastolic, and mean blood pressures were recorded for 20 minutes thereafter.

Morphometric Analysis of Tissue
The animals were euthanized with an overdose of a commercial intravenous barbiturate (Sleepaway, 10 mL by ear vein). The hearts were immediately removed and the coronary arteries fixated by pressure perfusion (100 mm Hg) with 10% neutral buffered formalin for 24 hours. After fixation, the stented coronary segments were dissected free, stent wires removed, and the vessels cut at 2-mm perpendicular intervals. The tissues were embedded and stained with hematoxylin-eosin and van Gieson’s elastin stain (Figure 1). The neointimal response was measured from the elastin-stained sections prepared for morphometric analysis in placebo (A) and high-dose (B) pigs. van Gieson elastin stain. Magnification ×25.

Immunostaining

Immunoactivity for ET-1 in stented coronary segments prepared for morphometric analysis in placebo (A) and high-dose (B) pigs. van Gieson elastin stain. Magnification ×25.

Figure 1. Representative examples of stented coronary segments prepared for morphometric analysis in placebo (A) and high-dose (B) pigs. van Gieson elastin stain. Magnification ×25.

was also measured at each wire site, and mean injury scores and neointimal responses were calculated for each stented coronary segment. Vessel size was measured by determining the area contained within the external elastic lamina.

Statistical Analysis
A sample size of 13 animals per group was chosen to allow detection of a projected difference in neointimal thickness of 0.1 mm at a power of 0.8. Statistical analysis was performed on injury and neointima at each wire site. Regression modeling was used to account for injury and the injury-dependent neointimal response. Three models were used to establish whether there were differences...
TABLE 1. Mean Blood Pressures at Days 0 and 28, Before and After Big-ET Injection, in Each Treatment Group Compared With Placebo

<table>
<thead>
<tr>
<th>Blood Pressure, mm Hg</th>
<th>Day 0</th>
<th>Day 28</th>
<th>After Big ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>112±21</td>
<td>113±12</td>
<td>144±14</td>
</tr>
<tr>
<td>Low dose</td>
<td>108±22</td>
<td>99±23*</td>
<td>127±28*</td>
</tr>
<tr>
<td>Mid dose</td>
<td>112±27</td>
<td>104±15</td>
<td>129±18*</td>
</tr>
<tr>
<td>High dose</td>
<td>108±15</td>
<td>99±11*</td>
<td>119±12*</td>
</tr>
</tbody>
</table>

ET indicates endothelin.

*P<0.05 vs placebo group.

in intercepts, slopes, or both intercepts and slopes across the four groups studied. This was performed as previously described.30 Briefly, the three regressions were tested by use of the following equations. Note that the variables labeled Gp establish the treatment group to either slope or intercept of neointima and injury. These models were as follows.


Differences between treatment groups at each injury level were analyzed by the Tukey-Kramer multiple comparisons t test.

Results

A total of 55 pigs underwent stent deployment in each of two randomly assigned major epicardial coronary arteries. Three animals (5.5%) died as a consequence of stent thrombosis within 24 hours of the procedure. The remaining 52 animals survived without complications until predetermined euthanasia at 28 days. This resulted in 13 pigs and 26 stented coronary segments per treatment group.

Resting Blood Pressures on Days 0 and 28, Before and After Big ET-1

The mean resting blood pressures on day 0 were similar in all four groups (Table 1). After 28 days of treatment with the ETα antagonist, the mean resting pressure in the low- and high-dose animals was significantly lower than in placebo animals (P<0.05). The average absolute reduction in mean blood pressure was 14 mm Hg. The lower mean blood pressure in the mid-dose group at this time point did not reach significance (P=0.07). The pressor response to big ET-1 was significantly blunted in all three treatment groups compared with placebo (P<0.05). The average absolute reduction in mean pressor response was 19 mm Hg and was greatest in high-dose animals. These findings suggest excellent ETα antagonism.

Arterial Injury Score and Neointimal Hyperplasia

In the placebo group, the mean injury score was 1.73±0.80, with mean neointimal thickness of 0.45±0.24 mm (Table 2, Figure 1). In comparison, the low-dose group had a similar mean injury score of 1.79±0.75, with a corresponding significantly reduced neointimal response of 0.36±0.22 mm (P<0.01). The mean injury score in the mid-dose animals was significantly more than in the placebo group (1.94±0.92; P<0.05). However, the neointimal hyperplasia associated with this injury was still less than in the placebo group, although it did not quite reach statistical significance (0.40±0.25 mm; P=0.05). In the high-dose pigs, the mean injury score was also significantly greater than in the placebo arm (1.93±0.73; P<0.05); despite this, the neointimal response was significantly less (0.37±0.26 mm; P<0.01). The ratio of mean neointimal thickness to injury score was 0.2, 0.2, and 0.19 in low-, mid-, and high-dose animals, respectively, compared with 0.26 in placebo. The average absolute reduction in neointimal thickness in the treatment groups was 0.06 mm. Similarly, the neointimal area (mm²) 28 days after coronary stenting was significantly less in low- and high-dose animals (P<0.01) and nonsignificantly less in mid-dose animals (P=0.07) compared with placebo. The average absolute reduction in neointimal area in the treatment groups was 0.5 mm². There were no significant differences between the three groups of treated animals regarding injury score or subsequent neointimal hyperplasia.

Figure 2 and Table 3 show the results of regression modeling. These results indicate that the intercepts differed significantly between all drug treatment groups and placebo, whereas the slopes did not.

Arterial Lumen and Vessel Areas

The significant reduction in neointimal hyperplasia observed translated into a significantly greater coronary lumen area (mm²) in the low-dose (3.79±1.39; P<0.05) and high-dose (4.12±1.75; P<0.01) groups compared with placebo animals (3.04±1.34) (Table 2). Twenty-eight days after the stenting, the average absolute gain in coronary in-stent lumen area in the low- and high-dose groups was 0.9 mm². Although the lumen area in the mid-dose group remained similar to that in

TABLE 2. Mean Arterial Injury Score and Corresponding NI Thickness and Area, Lumen Area, Percent Stenosis, and Total Vessel Area (Area Within the External Elastic Lamina) in Each Treatment Group Compared With Placebo

<table>
<thead>
<tr>
<th>Group</th>
<th>Injury</th>
<th>NI Thickness, mm</th>
<th>NI Area, mm²</th>
<th>Lumen Area, mm²</th>
<th>% Stenosis</th>
<th>Total Vessel Area, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>1.73±0.80</td>
<td>0.45±0.24</td>
<td>1.41±0.72</td>
<td>3.36±1.48</td>
<td>36±18</td>
<td>6.70±2.36</td>
</tr>
<tr>
<td>Low dose</td>
<td>1.79±0.75</td>
<td>0.36±0.22*</td>
<td>0.85±0.58*</td>
<td>4.21±1.61*</td>
<td>24±15*</td>
<td>6.88±1.84</td>
</tr>
<tr>
<td>Mid dose</td>
<td>1.94±0.92*</td>
<td>0.40±0.25</td>
<td>1.04±0.91</td>
<td>3.61±1.32</td>
<td>29±29</td>
<td>5.90±2.46</td>
</tr>
<tr>
<td>High dose</td>
<td>1.93±0.73*</td>
<td>0.37±0.37*</td>
<td>0.91±0.68*</td>
<td>4.38±1.58*</td>
<td>26±23*</td>
<td>7.14±1.92</td>
</tr>
</tbody>
</table>

NI indicates neointima.

*P<0.05.
the placebo group (2.96±1.53; \(P=0.42\)), there was a trend toward smaller vessel area (5.90±2.46) in this group compared with low-dose (6.88±1.84; \(P=0.08\)), high-dose (7.14±1.92; \(P=0.05\)), and placebo animals (6.70±2.36; \(P=0.14\)).

The coronary stenosis produced by neointimal thickening was correspondingly significantly less in low-dose (24±15%; \(P<0.05\)) and high-dose (26±23%; \(P<0.05\)) groups compared with placebo (36±18%), with absolute and relative reductions in luminal stenosis in all treated animals of 10% and 28%, respectively.

**Immunostaining**

ET-1 immunoreactivity was observed in uninjured porcine coronary artery only in the endothelium (Figure 3A). By comparison, ET-1 immunoactivity was found in the neointima of stented coronary segments, particularly at sites of strut injury (Figure 3B).

**TABLE 3. Regression Model Results for Determination of Differences of Neointimal Thickening**

<table>
<thead>
<tr>
<th>Regression model</th>
<th>Coefficient</th>
<th>(P) (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: (\text{Neointima} = \text{Constant} + \text{Injury} + \text{Gp2} + \text{Gp3})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>0.289</td>
<td>0.002</td>
</tr>
<tr>
<td>Injury</td>
<td>0.074</td>
<td>0.156</td>
</tr>
<tr>
<td>Gp2</td>
<td>-0.118</td>
<td>0.06</td>
</tr>
<tr>
<td>Gp3</td>
<td>-0.121</td>
<td>0.06</td>
</tr>
<tr>
<td>2: (\text{Neointima} = \text{Constant} + \text{Injury} + \text{Gp2} + \text{Gp3} + \text{Gp2} \times \text{Injury} + \text{Gp2} \times \text{Injury})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-0.01</td>
<td>0.939</td>
</tr>
<tr>
<td>Injury</td>
<td>0.279</td>
<td>0.003</td>
</tr>
<tr>
<td>Gp2</td>
<td>0.435</td>
<td>0.034</td>
</tr>
<tr>
<td>Gp3</td>
<td>0.211</td>
<td>0.181</td>
</tr>
<tr>
<td>Gp2 \times \text{Injury}</td>
<td>-0.371</td>
<td>0.006</td>
</tr>
<tr>
<td>Gp3 \times \text{Injury}</td>
<td>-0.235</td>
<td>0.037</td>
</tr>
<tr>
<td>3: (\text{Neointima} = \text{Constant} + \text{Injury} + \text{Gp2} \times \text{Injury} + \text{Gp3} \times \text{Injury})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>0.195</td>
<td>0.006</td>
</tr>
<tr>
<td>Injury</td>
<td>0.150</td>
<td>0.005</td>
</tr>
<tr>
<td>Gp2 \times \text{Injury}</td>
<td>-0.104</td>
<td>0.010</td>
</tr>
<tr>
<td>Gp3 \times \text{Injury}</td>
<td>-0.102</td>
<td>0.019</td>
</tr>
</tbody>
</table>

**Figure 2.** Mean injury score versus mean neointimal thickness (mm) regression lines. Note difference in intercepts of treated groups.

**Figure 3.** Representative examples of positive ET-1 immunostaining in an uninjured porcine coronary artery (A) compared with that of a stented coronary segment (B). Magnification ×75.

**Discussion**

Selective ET\(_A\) receptor antagonism, when given in a twice-daily oral formulation in this porcine model, significantly reduced neointimal hyperplasia in the first 28 days after coronary stenting. Low-dose (0.75 mg/kg) and high-dose (10.0 mg/kg) treatments were equally efficacious in inhibiting neointimal thickening. The high-dose animals demonstrated this significant attenuation of the neointimal response to coronary stenting despite sustaining a significantly greater initial injury than placebo animals. The mid-dose (3.75 mg/kg) animals also sustained a significantly greater coronary injury than placebo animals and demonstrated a nonsignificant \((P=0.05)\) reduction in neointimal hyperplasia. The reduction observed in neointimal hyperplasia in the low- and high-dose groups was associated with a significantly greater coronary lumen area compared with placebo, with no significant change in overall vessel area as delineated by external elastic lamina. In the mid-dose group, the coronary lumen area at 28 days showed no improvement compared with placebo, and these animals showed a trend toward reduced vessel area compared with placebo and the other two treatment groups.

The gain in arterial lumen area achieved by low- and high-dose drug was presumably secondary to the reduction in neointimal hyperplasia, because total vessel area did not change. The 0.9-mm\(^2\) gain in lumen area represents a 28% proportional gain in lumen compared with placebo. This result was achieved with only 28 days of treatment after
coronary stenting with significant arterial injury, only a single bolus of heparin, and no ticlopidine.

The increase in neointimal ET-1 immunoreactivity observed after coronary stenting in this porcine model is consistent with evidence that already implicates ET-1 in the pathogenesis of coronary restenosis. Interestingly, ET-1 staining after coronary stenting was concentrated in the neointima and was frequently seen at sites of neointima where stent struts were located. This finding provides motivation that the target of treatment, namely ET-1, is clearly present at arterial sites where treatment is necessary.

Metal stents by themselves do not inhibit the neointimal response to coronary injury but rather actually stimulate this process, with histological studies in pigs and intravascular ultrasound evaluation in patients identifying neointimal hyperplasia as the principal cause of in-stent restenosis. A recent histological study of specimens obtained by athectomy from in-stent restenosis in human peripheral arterial disease has confirmed the predominant role of smooth muscle cells in this process. Proliferative activity and apoptosis were documented in this study by staining with proliferating cell nuclear antigen and DNA nick-end labeling, respectively. This result identified smooth muscle cell proliferation as contributing to in-stent restenosis in humans.

Evidence suggests that embryonic endothelial cells can migrate to the subendothelial space and differentiate into vascular smooth muscle cells. Hence, it is conceivable that endothelium-derived cells may contribute to the smooth muscle cell proliferation observed in atherosclerotic and restenotic tissue while maintaining the capacity to secrete ET.

The vasoconstrictor, mitogenic, and proliferative properties of ET provide strong evidence for its role in coronary atherosclerosis. Endothelial cell injury is a critical initiating event in atherogenesis. The release of ET is stimulated by vessel injury and by atherogenic oxidized LDLs even when the endothelium remains intact. Human atherosclerotic plaque demonstrates a highly significant increase in both big ET and ET compared with histologically normal vessel, with dense binding of ET-1 observed in medial smooth muscle cells of normal and diseased aorta by autoradiography. ET-1 immunoreactivity is increased after coronary angioplasty in patients, both in the coronary sinus and in the distal segment of the injured artery. The level of reactivity correlates with the degree of mechanical stress applied to the arterial lesion.

After endothelial denudation in the rabbit carotid artery, tissue ET-1 levels increase significantly within 1 to 3 days. Despite almost complete endothelial regeneration after 4 weeks in this model, the tissue ET-1 level remains markedly higher than in control vessels. Balloon injury in the rat carotid model is associated with a 20-fold increase in levels of ETα receptor mRNA at 3 and 7 days after angioplasty. At 14 days, there was a corresponding increase in ET immunoreactivity, which was concentrated mainly in the neointima. The infusion of ET-1 in the rat carotid balloon injury model has been shown to worsen neointimal hyperplasia after mechanical injury. The neointimal hyperplasia observed in this model has been significantly reduced by nonselective and selective ETα receptor antagonism.

The homeostatic mechanisms that regulate vessel tone and the response to vessel injury seem to involve the counteracting forces of vasodilators, such as NO, and vasoconstrictors, such as angiotensin II and the ETs. Guanylate cyclase has been shown to have a mediator role in NO-induced apoptosis in vascular smooth muscle cells. The apoptosis induced by NO donor and cGMP analogue was directly antagonized by angiotensin II. The countervailing balance between such vasoactive substances may thus control cell growth and cell death. With the vasoconstrictor and mitogenic effects of ET-1 with ETα receptor blockade inhibited, the balance shifts in favor of NO and programmed cell death. This is one potential mechanism by which vascular smooth muscle cell and neointimal proliferation may be attenuated by selective ETα inhibition.

Neointima in pigs and humans contains extracellular matrix, as well as smooth muscle cells. This matrix constitutes the majority of restenotic neointima and contains primarily glycosaminoglycans and collagen. In porcine coronary arteries, both ET-1 and angiotensin II stimulate collagen synthesis by smooth muscle cells, with ET-1 acting as a direct agonist for collagen type I synthesis. The specific ETα receptor antagonist BQ123 significantly inhibited the stimulatory effects of ET-1 in an in vitro study. Furthermore, clear evidence of reduced collagen deposition was observed in pig iliac arteries treated with ABT127722.5, the racemate of ABT147627. Coronary balloon injury in the porcine model not only induces smooth muscle cell proliferation and collagen synthesis but also stimulates the proliferation and migration of adventitial myofibroblasts across the external elastic lamina toward the coronary lumen. Thus, ETα antagonism may reduce neointimal hyperplasia by attenuating the proliferation of adventitial myofibroblasts as well as the proliferation of vascular smooth muscle cells and formation of extracellular matrix.

The efficacy of the ETα receptor antagonism in reducing neointimal hyperplasia after coronary stenting in this porcine model further solidifies the evidence implicating ET-1 in the pathogenesis of coronary restenosis. Clinical studies using this oral selective ETα receptor antagonist may be warranted on the basis of the above results.

References

ET\textsubscript{A} Antagonism and Neointimal Hyperplasia


Selective ETA Receptor Antagonism Reduces Neointimal Hyperplasia in a Porcine Coronary Stent Model


Circulation. 1998;97:2551-2556
doi: 10.1161/01.CIR.97.25.2551

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
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:
http://circ.ahajournals.org/content/97/25/2551

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/