Beneficial Effects of Clopidogrel Combined With Aspirin in Reducing Cerebral Emboli in Patients Undergoing Carotid Endarterectomy

David A. Payne, FRCS; Chris I. Jones, MSc; Paul D. Hayes, FRCS; Matthew M. Thompson, MD; Nicholas J. London, MD; Peter R. Bell, MD; Alison H. Goodall, PhD; A. Ross Naylor, MD

Background—Postoperative thromboembolic stroke affects 2% to 3% of patients undergoing carotid endarterectomy (CEA) and is preceded by 1 to 2 hours of increasing cerebral embolization. Previous work has demonstrated that high rates of postoperative embolization are associated with increased platelet reactivity to adenosine 5'-diphosphate (ADP). Our hypothesis was that preoperative administration of the platelet ADP antagonist clopidogrel could reduce postoperative embolization.

Methods and Results—One hundred CEA patients on routine aspirin therapy (150 mg) were randomized to 75 mg clopidogrel (n=46) or placebo (n=54) the night before surgery. Platelet response to ADP was assessed by whole-blood flow cytometry. The number of emboli detected by transcranial Doppler within 3 hours of CEA was independently quantified. Time taken from flow restoration to skin closure was used as an indirect measure of the time to secure hemostasis. In comparison with placebo, clopidogrel produced a small (8.8%) but significant reduction in the platelet response to ADP (P=0.05) while conferring a 10-fold reduction in the relative risk of those patients having &gt;20 emboli in the postoperative period (odds ratio, 10.23; 95% CI, 1.3 to 83.3; P=0.01, Fisher’s exact test). However, in the clopidogrel-treated patients, the time from flow restoration to skin closure (an indirect marker of hemostasis) was significantly increased (P=0.04, Fisher’s exact test), although there was no increase in bleeding complications or blood transfusions.

Conclusions—This is the first study to show that a CEA patient’s postoperative thromboembolic potential can be significantly reduced by targeted preoperative antiplatelet therapy without increasing the risk of bleeding complications. (Circulation. 2004;109:1476-1481.)

Key Words: stroke ■ prevention ■ surgery, carotid ■ complications, postoperative ■ inhibitors, platelet aggregation

Despite a proven role in the management of symptomatic carotid artery disease, the paradox remains that 5% to 7% of patients will suffer a stroke after carotid endarterectomy (CEA).1,2 The vascular unit at Leicester has undertaken a sequence of studies aimed at reducing the operative risk after CEA. Intraoperative stroke, apparent on recovery from anesthesia, was reduced in the last 1200 patients from 4% to 0.25% by introducing a policy of intraoperative transcranial Doppler (TCD) monitoring and completion angioscopy.3,4 However, this failed to prevent postoperative stroke due to thrombosis of the endarterectomy zone, which continued to complicate 2.7% of CEAs.4

See p 1442

Five centers have now shown that patients destined to suffer a postoperative thrombotic stroke have a 1- to 2-hour period of increasing embolization before the neurological deficit becomes apparent.3,5-8 The actual number of patients with high-grade embolization is &lt;5%,4 but of these 30% to 60% will progress to thrombotic stroke.3,5 Postoperative TCD monitoring and selective administration of dextran-40 has been successful in reducing embolization and progression to stroke.9 However, this policy is expensive and labor intensive and is unlikely to be adopted into routine clinical practice. It would clearly be preferable to target appropriate pharmacotherapy from the outset.

Aspirin therapy has been shown to reduce the relative risk of thromboembolic stroke by 20% to 25%,10 yet 2% to 5% of patients will still suffer an ischemic stroke in the perioperative period.11 Aspirin inhibits only 1 of the several pathways of platelet activation. Failure to prevent thromboembolization in carotid surgery suggests that platelet activation through an
aspirin-insensitive pathway may be more important in promoting arterial thromboembolization in this setting. A recent prospective trial of patients undergoing CEA (standardized to aspirin before and after surgery) concluded that there was no correlation between the magnitude of embolization and the ability of aspirin to inhibit platelet aggregation induced by arachidonic acid.12 In these patients, aspirin resistance was not linked to failure of preventing embolization. However, a link was found between the level of the platelet response to adenosine 5'-diphosphate (ADP) and postoperative embolization. In patients with enhanced preoperative sensitivity to ADP, there were significantly greater numbers of postoperative emboli compared with the group of low ADP responders.12

The hypothesis underlying the present study was that preoperative administration of low-dose clopidogrel (an ADP receptor antagonist) should reduce ADP-mediated platelet activation, leading to a reduction in postoperative embolization.

**Methods**

**Study Design**

Approval was obtained from the Leicestershire Health Authority Research Ethics Committee for a double-blinded randomized controlled trial in consecutive patients scheduled for CEA. All patients were standardized to 150 mg of aspirin for 4 weeks before surgery. With the use of previously generated randomized computer codes, patients were then given either placebo or 75 mg of clopidogrel 12 hours before surgery. The appointed trial pharmacist held a secure record of all the randomization codes, whereas patients and all other personnel concerned with the study or patient care were blinded from the trial drug allocation.

Thirty-eight patients were excluded (Figure 1). Exclusion criteria included refusal to give consent (n=6); aspirin intolerance (n=1); absence of a transcranial window for TCD monitoring (n=10); emergency CEA (n=2); patients already on warfarin, dipyridamole, or clopidogrel therapy (n=15); vein bypass performed instead of CEA (n=2); and CEA performed under local anesthesia (n=2). Power calculations were performed on the basis of previous postoperative embolization rates,13 which suggested that randomizing a total of 100 patients would enable a 90% chance of detecting a 20% difference between the 2 groups.

**Blood Collection**

Blood samples were taken before administration of the trial drug and immediately before surgery. After the patient had rested supine for 20 minutes, a standardized phlebotomy technique with minimal stasis and clean venipuncture was used to minimize artifactual platelet activation.14 Blood was collected from the antecubital fossa via a 21-gauge butterfly needle into Vacutainer tubes (Becton Dickinson), with the first 3 mL of blood taken into EDTA (4.5 mg) and used to obtain a full blood count and mean platelet volume (AC T Diff Analyser, Coulter Electronics Ltd). Subsequent samples were taken into trisodium citrate (3.8% wt/vol) and processed immediately for platelet aggregometry and whole-blood flow cytometry.

**Platelet Aggregometry**

Platelet-rich plasma samples were prepared for aggregometry by centrifugation for 20 minutes at 900 rpm. Born-type aggregometry15 was used to assess platelet aggregation in response to arachidonic acid (2.5×10⁻¹⁰ mol/L; Sigma) with the use of a PAP4 aggregometer (BioData Corp). Aggregation was measured as the percentage of maximal aggregation performed over a 10-minute period.

**Whole- Blood Flow Cytometry**

Flow cytometric analysis was performed according to methods previously described.14,15 Briefly, fibrinogen binding to platelets was measured in unstimulated blood samples and in samples stimulated with ADP (1×10⁻⁶ mol/L; Sigma) by the binding of an FITC-conjugated rabbit antibody to fibrinogen (Dako Ltd) to the platelets. Samples were analyzed in a Beckman-Coulter MCL-XL flow cytometer (Beckman-Coulter Electronics Ltd). Fibrinogen binding is expressed as the percentage of platelets found positive for binding of the fluorescent antibody. An unstimulated sample incubated in the presence of 2.5×10⁻⁶ mol/L EDTA (to prevent fibrinogen binding to glycoprotein IIb/IIIa) provided a negative control.

**Operation and Monitoring**

All patients underwent a standardized CEA with the use of normocarbic, normocapnic general anesthesia. Systemic heparinization (5000 U) was administered before insertion of the Pruitt-Inahara shunt. Continuous TCD monitoring of the blood flow velocity in the middle cerebral artery was performed for the duration of the operation and for 3 hours after surgery. All TCD waveform data were stored on digital audiotape for offline analysis. Postoperative embolization was quantified with the use of standardized consensus criteria.16 Any patient suffering high-grade embolization (>25 emboli in any given 10-minute period) was started on intravenous dextran-40 (Pharmacia Ltd) according to the departmental protocol.17 All personnel involved with the trial were blinded to the nature of the patients' current drug therapy.

Any new neurological deficit lasting for >24 hours in the first 30 days was classified as a stroke. The timing of any new event was recorded (intraoperative versus postoperative). The severity of any detected stroke was evaluated by a neurologist and graded according to the Oxfordshire Handicap Scale. Any patient suffering a perioperative event was investigated by duplex ultrasound, TCD, and CT scanning.
by guest on April 16, 2017 http://circ.ahajournals.org/ Downloaded from

Whitney

A 10-fold reduction in the relative risk (odds ratio, 10.23; 95% confidence interval, 1.3 to 83.3; P=0.01, Fisher’s exact test). This effect was seen throughout the spectrum from low- to high-grade embolization; importantly, within a 3-hour period of monitoring, no patient taking clopidogrel had >25 emboli detected.

Two patients in the placebo group required dextran-40 to control high-grade embolization. No patients in the clopidogrel arm received dextran therapy.

Whole-Blood Flow Cytometric Analysis
Figure 3 demonstrates that clopidogrel produced a small (8.8%) but significant reduction in platelet fibrinogen binding in response to ADP, which was nonetheless significant compared with the placebo group (66.76±2.9% versus 75.52±2.7%; P=0.03). Before drug administration, there was no significant difference in ADP response between the 2 groups (73.2±2.9% versus 73.6±3.1%; P=0.73). There was also no significant difference in fibrinogen binding to unstimulated platelets between the 2 drug groups before or after subjects took the trial drug before surgery (before drug: 2.0% versus 2.0%; after drug: 3.6% versus 3.9%; P>0.05).

Ex Vivo Platelet Aggregation
Aspirin inhibition was confirmed by measuring platelet aggregation in response to arachidonic acid (2.5×10⁻⁶ mol/L). Platelet aggregation was noted to be minimal in both arms of the trial (3.8±3.5 for clopidogrel versus 4.3±4.0 for placebo; P=0.51, t test). None of the patients was deemed to be “aspirin resistant,” defined as platelet aggregation in response to arachidonic acid >20% of maximum.18

Hemostatic Function
An indirect measure of hemostasis was undertaken by measuring the time from restoration of blood flow to closure of the neck wound. Other indirect markers of blood loss included neck hematomas, wound drainage volume, and the number of patients returning to the operating room for exploration of bleeding.

Statistical Analysis
Discrete data were analyzed in SPSS with the use of contingency tables (Fisher’s exact test), and continuous data were analyzed with the use of a 2-tailed Student t test (platelet analysis) or Mann-Whitney U test (patient variables). Probability values <0.05 were considered statistically significant.

Results
Epidemiology
Between February 2000 and October 2001, 138 patients underwent CEA at the Leicester Royal Infirmary. Of these, 38 patients (27.5%) were excluded (see earlier), and 100 patients (72.5%) were randomized to either clopidogrel (n=46) or placebo (n=54) (Figure 1). The uneven distribution in the 2 groups arose because 4 patients who required staged bilateral CEAs received the same therapy for both procedures.

Both groups were well matched, with no significant difference in age, sex, weight, atherosclerotic risk factors, or presenting complaint (Table 1).

Postoperative Embolization
To enable meaningful statistical analysis, an arbitrary cutoff point of 20 emboli was used. The magnitude of embolization in the first 3 hours after surgery was significantly reduced in the clopidogrel group (1 of 46; 2.2%) compared with patients receiving placebo (10 of 54; 18.5%) (Figure 2), representing a 10-fold reduction in the relative risk (odds ratio, 10.23; 95% CI, 1.3 to 83.3; P=0.01, Fisher’s exact test). This effect was seen throughout the spectrum from low- to high-grade embolization; importantly, within a 3-hour period of monitoring, no patient taking clopidogrel had >25 emboli detected.

Two patients in the placebo group required dextran-40 to control high-grade embolization. No patients in the clopidogrel arm received dextran therapy.

Whole-Blood Flow Cytometric Analysis
Figure 3 demonstrates that clopidogrel produced a small (8.8%) but significant reduction in platelet fibrinogen binding in response to ADP, which was nonetheless significant compared with the placebo group (66.76±2.9% versus 75.52±2.7%; P=0.03). Before drug administration, there was no significant difference in ADP response between the 2 groups (73.2±2.9% versus 73.6±3.1%; P=0.73). There was also no significant difference in fibrinogen binding to unstimulated platelets between the 2 drug groups before or after subjects took the trial drug before surgery (before drug: 2.0% versus 2.0%; after drug: 3.6% versus 3.9%; P>0.05).

Ex Vivo Platelet Aggregation
Aspirin inhibition was confirmed by measuring platelet aggregation in response to arachidonic acid (2.5×10⁻⁶ mol/L). Platelet aggregation was noted to be minimal in both arms of the trial (3.8±3.5 for clopidogrel versus 4.3±4.0 for placebo; P=0.51, t test). None of the patients was deemed to be “aspirin resistant,” defined as platelet aggregation in response to arachidonic acid >20% of maximum.18

Figure 3. Fibrinogen binding to platelets in response to ADP (1×10⁻⁶ mol/L) before and after clopidogrel but before surgery. Binding was analyzed in whole blood from patients treated with aspirin (150 mg) alone (○) or aspirin (150 mg) plus clopidogrel (75 mg) (●) 12 hours before testing. Values are mean±SEM.

Table 1. Demographic Breakdown of Trial Patients Randomized to Aspirin (150 mg) + Placebo or Aspirin (150 mg) + Clopidogrel (75 mg)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (n=54)</th>
<th>Clopidogrel (n=46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, median±SD</td>
<td>69±8.5</td>
<td>68±8.9</td>
<td>0.75</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>40 (74)</td>
<td>36 (78)</td>
<td>0.65</td>
</tr>
<tr>
<td>Female</td>
<td>14 (26)</td>
<td>10 (22)</td>
<td></td>
</tr>
<tr>
<td>Weight, kg, median±SD</td>
<td>76±11.0</td>
<td>77±13.1</td>
<td>0.32</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>37 (69)</td>
<td>35 (76)</td>
<td>0.50</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>17 (32)</td>
<td>17 (37)</td>
<td>0.77</td>
</tr>
<tr>
<td>Statin treatment, n (%)</td>
<td>24 (44)</td>
<td>22 (48)</td>
<td>1.0</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>10 (19)</td>
<td>12 (26)</td>
<td>0.47</td>
</tr>
<tr>
<td>Claudication, n (%)</td>
<td>10 (19)</td>
<td>9 (20)</td>
<td>1.0</td>
</tr>
<tr>
<td>Angina, n (%)</td>
<td>9 (17)</td>
<td>8 (17)</td>
<td>1.0</td>
</tr>
<tr>
<td>Myocardial infarction, n (%)</td>
<td>9 (17)</td>
<td>10 (22)</td>
<td>0.44</td>
</tr>
<tr>
<td>Asymptomatic, n (%)</td>
<td>11 (20)</td>
<td>5 (11)</td>
<td>0.55</td>
</tr>
<tr>
<td>Amaurosis fugax/TIA, n (%)</td>
<td>33 (61)</td>
<td>33 (72)</td>
<td></td>
</tr>
<tr>
<td>Stroke, n (%)</td>
<td>10 (19)</td>
<td>8 (17)</td>
<td></td>
</tr>
<tr>
<td>Carotid stenosis, %±SD</td>
<td>80±8.8</td>
<td>81±8.6</td>
<td>0.60</td>
</tr>
</tbody>
</table>

TIA indicates transient ischemic attack.

Figure 2. Postoperative emboli during 3 hours of monitoring after surgery, ranked in ascending order for patients randomized to either aspirin (150 mg) plus placebo or aspirin (150 mg) plus clopidogrel (75 mg).
Table 2. Markers of Blood Loss for Trial Patients Randomized to Aspirin (150 mg) + Placebo or Aspirin (150 mg) + Clopidogrel (75 mg)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (n=54)</th>
<th>Clopidogrel (n=46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck drainage, mL, median±SD</td>
<td>72±115</td>
<td>52±50.3</td>
<td>0.65</td>
</tr>
<tr>
<td>Hematomas, n (%)</td>
<td>3 (6)</td>
<td>6 (13)</td>
<td>0.47</td>
</tr>
<tr>
<td>Transfusion, n (%)</td>
<td>4 (7)</td>
<td>3 (7)</td>
<td>1.0</td>
</tr>
<tr>
<td>Reexploration, n (%)</td>
<td>3 (6)</td>
<td>5 (11)</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Platelet Count and Mean Platelet Volume
There was no significant difference in platelet count between patients receiving placebo or clopidogrel either at baseline (259.3±68.6 versus 250.0±81.6; P=0.68), at 12 hours after taking the drug (247.0±64.1 versus 233.9±55.4; P=0.35), or at the end of surgery (228.3±59.0 versus 212.0±71.7; P=0.44). There was also no significant difference in mean platelet volume for the 2 treatment groups at any time point (P>0.05).

Hemostatic Function
Despite a significant reduction in postoperative embolization in patients taking clopidogrel, the reduction in ADP-mediated platelet activation did not lead to a significant increase in markers of blood loss (Table 2) compared with the placebo group (P>0.05 in all cases). Blood loss, as measured by neck drains, neck hematomas, or those patients requiring blood transfusion, was not significantly greater in the clopidogrel group. However, when we considered the length of time taken from restoration of blood flow to skin closure as an indirect marker of hemostasis, patients randomized to clopidogrel took significantly longer to secure hemostasis (Figure 4). For closure times ≥40 minutes, the percentage of patients requiring additional closure time was 30% in the clopidogrel group versus 8% in the placebo group (P=0.004, Fisher’s exact test).

Perioperative Morbidity and Mortality
No deaths occurred during the trial, although 1 patient in each group suffered a stroke (placebo group: intraoperative stroke; clopidogrel group: hyperperfusion-related stroke on day 5).

Discussion
Postoperative stroke was previously assumed to follow undiagnosed technical error. However, in patients reexplored for postoperative neurological deficit after completion angiography, a platelet-rich thrombus was invariably found adherent to an otherwise normal endarterectomy zone and not the patch. This suggested that it might be the patients’ inherent platelet activity that determined those at greatest risk of postoperative thrombotic stroke and not technical error.

Hayes et al. have shown that in patients undergoing CEA, the preoperative response of platelets to ADP was predictive of postoperative embolization, concluding that platelet ADP-receptor antagonism could prevent perioperative cerebral embolization. As a follow-up to this work, we have shown that a single dose of clopidogrel (75 mg) given the night before surgery reduces postoperative embolization through a small but significant reduction in platelet ADP function. Interestingly, this reduction in embolization did not translate into a significant increase in markers of blood loss, although neck closure times were considerably lengthened in several patients taking clopidogrel. In the placebo group, high sensitivity to ADP preoperatively was predictive of high rates of embolization after surgery, confirming our previous findings that ADP-mediated platelet activation is important in the pathogenesis of postoperative embolization.

Much debate has centered on the role of TCD in detecting embolization after carotid surgery. Concerns have centered on the sensitivity of TCD in detecting embolization and the specificity in distinguishing between artifact or gaseous or particulate emboli. Dual-gating techniques from a number of centers have conclusively proven Doppler ultrasound to be capable of differentiating emboli from artifacts, whereas a prospective cohort study from our unit demonstrated that post-CEA emboli are exclusively particulate.

In the present study we used postoperative embolization as a surrogate outcome measure for thromboembolic stroke. The need for an alternative end point arises because of the low incidence of stroke after carotid surgery, estimated to be from 2% to 5%. Our data have suggested that approximately 50% of patients will have ≥1 emboli detected during the 3-hour period of postoperative TCD monitoring. Of these, <5% will undergo high-grade embolization, defined as ≥25 emboli in a given 10-minute period. It has been shown independently and conclusively by 5 different research centers worldwide that in the postoperative period, increasing rates of embolization are seen before manifestation of a neurological deficit. If high embolization rates are detected by TCD monitoring, a therapeutic strategy of administering intravenous dextran-40 has been shown to both reduce high-grade postoperative embolization and prevent thromboembolic stroke, providing further evidence of the important association between these 2 events.

After carotid surgery, high embolization rates were still detected in 5 patients randomized to placebo. It appears that aspirin alone, inhibiting only 1 platelet activation pathway, will not prevent embolization resulting from surgery. In the present study, preoperative tests showed that aspirin was effective in inhibiting platelet activation in response to arachidonic acid in all subjects. None of these differences...
could therefore be attributed to “aspirin resistance,” again confirming previous observations in patients undergoing CEA who have been treated with aspirin.12

Although aspirin inhibits the generation of thromboxane A2 by blocking COX-1, clopidogrel exerts its antiplatelet effect by a different mechanism, blocking the platelet P2Y12 ADP receptor.22 The benefits of clopidogrel over aspirin have already been established for cardiovascular events in CAPRIE,23 in which clopidogrel was associated with significantly fewer events than aspirin alone. In the acute setting of intravascular intervention, the CLASSICS trial24 has shown conclusively that the combination of clopidogrel and aspirin had increased benefit over aspirin alone in reducing vascular ischemic events without increasing the risk of bleeding complications. In this study the addition of a single tablet of clopidogrel produced a modest although significant reduction in the platelet response to ADP and a significant reduction in embolization.

This adds further evidence to the benefits of combination antiplatelet therapy but raises an important issue. Although reduction of platelet activation via ADP would appear to be crucial in reducing carotid embolization, it is important to retain sufficient platelet function to achieve adequate hemostasis. Maximal inhibition of platelet aggregation is not an option in open vascular surgery in which hemorrhagic complications can lead to significant morbidity and mortality. The dosing regimen used in the study was selected from previous evidence, which compared the effects of administering 2 different doses of clopidogrel in combination with aspirin on bleeding time and platelet function.25 Although higher doses of clopidogrel (300 mg) and aspirin conferred maximal platelet inhibition, it came at a cost of a significant increase in bleeding time. In the present study, although the lower, single dose of clopidogrel (75 mg) given >12 hours before surgery resulted in a modest reduction in platelet ADP response in vitro, it did not lead to a significant increase in markers of blood loss or the need for blood product transfusion (Table 2). There was, however, a nonsignificant increase in neck hematoma and reexploration of the neck wound for bleeding in patients taking clopidogrel. The validity of this trend remains to be seen because this study was not powered to detect such differences. It is clear from our results that the neck closure times are increased significantly in the clopidogrel group and represent an increase in the time taken to secure hemostasis of the neck wound. Despite the fact that the majority of patients in the trial required >30 minutes to control bleeding, 10% of patients on clopidogrel required >60 minutes to complete neck closure. Hemostasis in these circumstances was ultimately achieved by a combination of gentle pressure over the wound and packing Surgicel (Ethicon Ltd) around the patch suture line. None of these patients required reexploration for bleeding.

The response to ADP varies considerably between individuals, and evidence is accumulating that this may relate to the amount of clopidogrel needed to inhibit platelets in vivo.25,26 Whether surrogate in vitro markers of platelet response to ADP could be incorporated into a therapeutic dosing strategy needs careful evaluation.

Inhibiting ADP-induced platelet activation and postoperative embolization represents a new and exciting therapeutic strategy in reducing thromboembolic events after CEA and potentially any procedure in which the endothelium is damaged. To determine whether the reduction in postoperative embolization translates into a reduction in stroke would require a multicenter randomized trial of patients on aspirin randomized to either placebo or clopidogrel. If successful, TCD monitoring and the use of a trained technician may no longer be required, offering a strategy that could be used by all vascular units irrespective of size and monitoring facilities. First, however, further work needs to be undertaken to determine whether an even lower dose of clopidogrel would confer a similar reduction in embolization while reducing the time to achieve hemostasis.

Acknowledgments

This study was supported by the UK Stroke Association, University Hospitals of Leicester research fellowship, and Bristol-Myers Squibb.

References


Beneficial Effects of Clopidogrel Combined With Aspirin in Reducing Cerebral Emboli in Patients Undergoing Carotid Endarterectomy

David A. Payne, Chris I. Jones, Paul D. Hayes, Matthew M. Thompson, Nicholas J. London, Peter R. Bell, Alison H. Goodall and A. Ross Naylor

_Circulation._ 2004;109:1476-1481; originally published online March 8, 2004;
doi: 10.1161/01.CIR.0000121739.05643.E6

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 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/109/12/1476

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/