Novel Oral Formulation of Paclitaxel Inhibits Neointimal Hyperplasia in a Rat Carotid Artery Injury Model

Dong-Woon Kim, MD; Jin-Sook Kwon, DVM; Young-Gyu Kim, MD; Maeng Sup Kim, PhD; Gwan-Sun Lee, PhD; Tae-Jin Youn, MD; Myeong-Chan Cho, MD

Background—Paclitaxel has been shown to inhibit vascular smooth muscle cell migration and proliferation contributing to neointimal formation. This study tested whether novel oral formulations of paclitaxel can prevent neointimal formation in a rat carotid artery injury model.

Methods and Results—Oral formulations of paclitaxel (0, 5, 7.5, or 10 mg/kg) were administered to 40 rats by gavage for 5 days after injury. The peak plasma levels of paclitaxel administered at 5, 7.5, and 10 mg/kg were 61 ± 16, 89 ± 22, and 108 ± 28 nmol/L, respectively. Treatment effects were assessed 11 days after injury. The angiographic minimum luminal diameters of the oral paclitaxel groups treated at 5, 7.5, and 10 mg/kg were 6.28 ± 2.09, 6.97 ± 1.79, and 7.97 ± 1.57 AU, and these were significantly larger than that of the control group (4.67 ± 1.45 AU). The oral paclitaxel groups (5, 7.5, 10 mg/kg; 0.05 ± 0.05, 0.04 ± 0.03, 0.05 ± 0.03 mm²) showed significant neointimal formation reductions versus the control group (0.13 ± 0.05 mm²). All rats survived to study completion. Only 2 animals in the 10 mg/kg group experienced weight loss (~10%) and loose stools between 4 and 6 days after injury. All other animals appeared healthy during the study. For comparison purposes, intraperitoneal formulations of paclitaxel (0 or 2 mg/kg) were administered by injection to 15 rats. We confirmed that the intraperitoneal administration of paclitaxel also effectively inhibited neointimal formation.

Conclusions—Oral formulations of paclitaxel provide an effective means of inhibiting proliferative response to vascular injury in the rat. Thus, oral formulations of paclitaxel may prevent human restenosis without significant toxicity. (Circulation. 2004;109:1558-1563.)

Key Words: paclitaxel ■ angioplasty ■ restenosis

Paclitaxel was found to interfere with vascular smooth muscle cell (VSMC) migration and proliferation at nanomolar levels in vitro. In vivo, paclitaxel inhibited neointimal formation in a rat carotid artery after endothelial denudation injury. Moreover, this effect occurred at plasma paclitaxel levels ~100 to 1000 times lower than the concentrations to treat neoplasms. Peak levels achieved in this model were ~50 to 60 nmol/L. Therefore, paclitaxel may be of therapeutic value in preventing human restenosis with minimal toxicity, but the intravenous infusion of commercial formulations of paclitaxel containing Cremophor EL is associated with the risk of hypersensitivity reactions and is inconvenient because of long infusion rates over periods of 3 to 24 hours.

The oral bioavailability of paclitaxel is very low. This is because of efficient transport of the drug by the intestinal drug efflux pump p-glycoprotein. It is well known that inhibitors of p-glycoprotein such as cyclosporine and verapamil enhance the oral bioavailability of paclitaxel. However, the usefulness of cyclosporine is limited by its immunosuppressive activity. Verapamil, a calcium channel blocker, is another extensively characterized modulator of p-glycoprotein, which is recognized as a significant factor in oral bioavailability and multidrug resistance (MDR). However, the usefulness of verapamil is also limited, because the plasma concentrations required to reverse MDR resulted in cardiac toxicity. KR-30031 (1-3-[2-(3,4-dimethoxyphenyl)ethyl]methylamino)propyl]-4,5-dimethoxyindan-1-carbonitrile, Figure 1) is a verapamil analogue with a p-glycoprotein inhibitory effect and is free of adverse cardiovascular effects (Korea Patent No. 10-0245981).

We recently developed an oral formulation of paclitaxel using KR-30031. It is expected that the oral absorption of paclitaxel can prevent fibroproliferative reactions, including restenosis after arterial injury.

Methods

Formulations of Paclitaxel

Paclitaxel (99.0% as anhydrous base) and KR-30031 (>99.0% by high-performance liquid chromatography [HPLC]) were synthesized at the Hanmi Pharmaceutical Co Ltd Central Research Institute, Korea. The following agents were purchased: Transcutol P (Gatte-
The novel oral formulation of paclitaxel was prepared by dissolving 12.8 mg of paclitaxel and 12.8 mg of KR-30031 in 1 mL of a surfactant mix composed of Transcutol P, Cremophor EL, Tween 80, dl-α-tocopheryl acetate, and ethyl linoleate (4.0:2.3:2.7:1.5:1.0, vol/vol/vol/vol/vol). Also, an oral vehicle was prepared by dissolving 12.8 mg of KR-30031 in 1 mL of this surfactant mix.

Intraperitoneal formulations were prepared as previously described. Briefly, 2 mg/kg body wt paclitaxel was dissolved in vehicle (13.4 mL/kg body wt of 1:2:2:165 DMSO/Cremophor EL–dl-surfactant mix composed of Transcutol P, Cremophor EL, Tween 80, dl-α-tocopheryl acetate, and ethyl linoleate (4.0:2.3:2.7:1.5:1.0, vol/vol/vol/vol/vol)).

**Surgical Procedures and Treatment Groups**

Twelve- to 14-week-old male Sprague-Dawley rats (Charles River Laboratory, Japan) weighing 300 g were fed a normal chow diet and given water ad libitum. All protocols were approved by the Chungbuk National University Animal Care and Use Committee.

Animals were anesthetized with an intraperitoneal injection of ketamine (50 mg/kg) and xylazine (6.7 mg/kg), and the right carotid artery was surgically exposed. A 2F Fogarty balloon embolectomy catheter (Baxter) was advanced along the length of the common carotid artery and retracted 3 times under mild balloon inflation pressure.

The oral treatment groups were divided into 4 groups: control (vehicle alone); low dose (5 mg/kg); intermediate dose (7.5 mg/kg); and high dose (10 mg/kg). Oral formulations of paclitaxel were given by gavage just before injury and daily for the next 4 days. Control animals were treated with vehicle alone (equivalent to the amount administered to the high-dose group).

There were 2 intraperitoneal treatment groups. Animals were treated with paclitaxel (2 mg/kg) or vehicle alone by intraperitoneal injection 2 hours after injury and then daily for the next 4 days.

**Measurement of In Situ VSMC Proliferation**

The effect of oral paclitaxel versus vehicle on in situ VSMC proliferation was measured by bromodeoxyuridine (BrdU) incorporation on day 2 after injury. Briefly, oral paclitaxel (5 and 10 mg/kg)– and oral vehicle–treated rats were injected subcutaneously with BrdU (30 mg/kg) at 30, 38, and 46 hours after injury. The carotid artery sections were harvested at 48 hours after injury, and histological sections were incubated with mouse anti-BrdU monoclonal antibodies (Boehringer Mannheim). The fraction of BrdU-positive medial VSMC nuclei per cross section was compared between the oral paclitaxel (5 and 10 mg/kg) and oral vehicle groups.

**Measurement of Plasma Paclitaxel**

Blood samples for oral administration were collected at 0.5, 1, 2, 3, 5, and 12 hours after drug administration. Five animals were used per time point in all experiments.

The blood samples (200 μL) were mixed with 200 μL of acetonitrile. After Vollex mixing, the mixture was centrifuged in an Eppendorf microvial (5417R, Eppendorf AG) for 5 minutes at 10,000 rpm and filtered through a 0.22-μm polyvinylidene difluoride membrane filter (Micropore No. SLGV013NL). The supernatant fraction (50 μL) was injected into an HPLC system incorporating a column switching system (Semi-micro HPLC system, SL2-2 model, Shiseido). The column switching system was composed of an analytical column (Capcell Pak C18 UG120 5 μm, 1.5×250 mm, Shiseido), a precolumn (Capcell Pak MF Ph-1, 4.6×10 mm, Shiseido), and a concentration column (Capcell Pak C18 UG120, 5 μm, 2.0×35 mm, Shiseido).

**Hematological and Blood Chemistry Assessment**

Blood samples from 3 to 4 animals per group were collected just before angiography, and complete blood counts were performed with an automated hematology analyzer (Sysmex NE-8000, Sysmex). Blood chemistry (glucose, BUN, creatinine, uric acid, total protein, albumin, AST, ALT, alkaline phosphatase) was also analyzed by computerized coronary angiography (DCI Videodensitometry, Phillips).

**Morphometric Analysis**

After the angiography, carotid arteries were perfusion-fixed with 10% buffered formalin. Carotid artery sections (5 μm) were stained with hematoxylin-eosin, and morphometric analysis was performed using 3 individual sections from the middle of each injured arterial segment by an investigator blind to the experimental procedure. The MLDs were measured using the APO-2000 system (Aperio). Cross-sectional areas (A_{intima} and A_{media}), area ratios (A_{intima}/A_{media}), and percent area stenosis (%) were analyzed and calculated using the Scion Image System (version 1.01).

**Histopathological Assessment of the Intestine**

Intestines were removed 11 days after surgery and fixed with 10% buffered formalin. Twenty animals (4 controls, 4 low doses, 4 intermediate doses, and all high doses) were evaluated. Intestinal sections (4 μm) were stained with hematoxylin-eosin, and histopathological evaluations were performed using 3 sections from different parts of the intestine.

**Statistical Analysis**

The results are expressed as mean±SD. Statistical comparisons between 2 groups were made using the 2-tailed, unpaired Student’s t test, whereas comparisons among oral paclitaxel–treated groups (4 groups) were analyzed using a 1-way ANOVA followed by Dunn’s post hoc test. A probability value of <0.05 was considered significant.

**Results**

Fifty-five rats were used for the experiments. Forty rats were used for the oral paclitaxel experiment, involving 4 treatment groups: 13 controls (vehicle alone), 9 at the low dose (5 mg/kg), 10 at the intermediate dose (7.5 mg/kg), and 8 at the high dose (10 mg/kg). Fifteen rats were used for the intraperitoneal paclitaxel experiment involving 2 treatment groups: 9 in the control group (vehicle alone) and 6 in the treatment group (2 mg/kg).
TABLE 1. Pharmacokinetic Parameters of Paclitaxel After Administration of Oral Formulation

<table>
<thead>
<tr>
<th>Paclitaxel Dose, mg/kg</th>
<th>AUC&lt;sub&gt;0–12h&lt;/sub&gt;, ng · h/mL</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;, nmol/L</th>
<th>t &gt;50 nmol/L, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>387±88</td>
<td>61±16</td>
<td>0.5±0.6</td>
</tr>
<tr>
<td>5</td>
<td>506±156</td>
<td>89±22</td>
<td>3.8±1.5</td>
</tr>
<tr>
<td>7.5</td>
<td>660±166</td>
<td>108±28</td>
<td>5.6±1.9</td>
</tr>
<tr>
<td>10</td>
<td>220±194</td>
<td>156±66</td>
<td>156±660</td>
</tr>
</tbody>
</table>

AUC<sub>0–12h</sub> (ng · h/mL) indicates area under the concentration-time curve; C<sub>max</sub>, maximal drug concentration; and t >50 nmol/L, h, time above concentration of 50 nmol/L.

All rats survived to study completion. In the high-dose oral paclitaxel group, which registered peak plasma levels of 108±28 nmol/L, 2 of the 8 rats experienced weight loss (~10% body weight) and loose stools between 4 and 6 days after injury. Subsequently, these 2 animals appeared well. All other animals appeared healthy during the course of the study.

Measurement of In Situ VSMC Proliferation
In vivo medial VSMC proliferation (assessed by in situ BrdU labeling) was inhibited in the low- and high-dose oral paclitaxel–treated groups at 2 days after injury (oral control versus low dose, 28.7±7.3% versus 10.7±4.8%; oral control versus high dose, 28.7±7.3% versus 14.1±4.9%; n=3 per group, P<0.01).

Measurement of Plasma Paclitaxel
The plasma AUC<sub>0–12h</sub> and C<sub>max</sub> of paclitaxel after administration of the oral formulation increased in a dose-dependent manner. The maximum plasma concentration of paclitaxel was reached within 2 to 3 hours of drug administration (Table 1).

Carotid Angiography
In the control angiogram, a distinct narrowing of the injured right carotid artery was evident. Luminal narrowing was markedly reduced in the oral paclitaxel–treated groups (Figure 2). The MLD of the injured carotid artery was compared with the luminal diameter of the uninjured left carotid artery (set at 10 arbitrary units [AU]). The MLDs of the oral paclitaxel–treated groups (5 mg/kg, 6.28±2.09 AU; 7.5 mg/kg, 6.97±1.79 AU; 10 mg/kg, 7.97±1.57 AU) were significantly larger than that of the oral control (4.67±1.45 AU). The MLD of the intraperitoneal paclitaxel–treated group (5.58±1.51 AU) was also found to be significantly larger than that of the intraperitoneal control (3.38±1.31 AU).

Morphometric Analysis
The oral paclitaxel–treated groups showed a significant reduction in neointimal formation compared with the oral control. The area ratios and percent stenosis of oral paclitaxel–treated groups were also reduced. However, the medial area was unchanged, suggesting that the medial wall can retain its integrity despite the administration of paclitaxel (Figure 3, Table 2).

Hematological Profiles
After 5 days of oral paclitaxel treatment at the high dose (10 mg/kg), 2 of 6 animals showed reduced peripheral white blood cell (WBC) counts (3.17±0.70×10<sup>3</sup>/μL; neutrophil count, 1.75±0.48×10<sup>3</sup>/μL). WBC counts of the other 4 animals were within normal limits (8.99±2.18×10<sup>3</sup>/μL; neutrophil count, 6.08±1.31×10<sup>3</sup>/μL). Five days after oral paclitaxel at 0 mg/kg (n=4) or 5 mg/kg (n=4), WBC counts were also within normal limits. At the end of the experiment, the hematological profiles of the oral paclitaxel experiment groups (n=3 to 4 per each group) were also within normal limits.

Blood Chemistry
Blood chemistries of the oral paclitaxel groups were also analyzed at days 5 and 11 after injury. All values (n=4 to 6 per each group) were within normal limits.

Histopathological Assessment of the Intestine
Mucosal structures were well preserved, and inflammatory cell infiltration was not increased in any sample (data not shown).

Discussion
Paclitaxel represents an appropriate therapeutic choice for the successful treatment of restenosis because of its effects on...
cellular processes, including enhanced potency in inhibiting VSMC migration and proliferation, and its proven efficacy in preventing restenosis. In terms of systemic therapy, the intraperitoneal application of paclitaxel in a rat carotid artery injury model showed that a significant (70%) reduction in neointimal proliferation could be achieved,¹ and in terms of local application, paclitaxel-coated coronary stents were found to inhibit neointimal hyperplasia in a porcine model of coronary restenosis 4 weeks after implantation.¹⁰ Clinical trials of paclitaxel-coated stents also have shown promise.¹¹,¹²

However, animal studies of paclitaxel-coated stents, although demonstrating efficacy, uncovered local cytotoxic drug effects and delayed neointimal healing.¹⁰,¹³ The selection of the ideal paclitaxel dosages to prevent neointimal proliferation while allowing healing and endothelialization is difficult because of the narrow therapeutic margin of paclitaxel and different release kinetics of stent coatings.¹⁰–¹⁴ Although human clinical trials with both paclitaxel- and sirolimus-coated stents are currently very promising, the long-term duration of clinical efficacy still remains to be solidly demonstrated.¹¹,¹²,¹⁵ Pending the results of definitive long-term clinical trials, late neointimal “catch-up” after stenting remains at least a potential adverse outcome, and thus, there could be a need for later adjunctive treatment that would be most conveniently delivered orally. Also, convenient systemic dosing could allow a more “uniform” treatment of multiple lesions in separate locations, as well as in patients who are not good candidates for stents because of small-vessel disease, vessel tortuosity, or complicating medical conditions.

Because there was no information on how much paclitaxel would be effective when applied orally in rats, results obtained from an intraperitoneal paclitaxel delivery study in the rat were used as a guide to the selection of an appropriate dosing range.¹ Peak blood levels achieved in this model were ≈50 to 60 nmol/L.¹ On the basis of the results of this study, we selected 3 oral paclitaxel–treated groups with peak blood levels >50 nmol/L. Thus, 4 groups were established, representing oral control (vehicle alone) and low (5 mg/kg), intermediate (7.5 mg/kg), and high (10 mg/kg) paclitaxel doses. The peak plasma concentrations of paclitaxel at the low, intermediate, and high dose were 61 ± 16, 89 ± 22, and 108 ± 28 nmol/L, respectively. The plasma concentration of paclitaxel in this experiment was significantly lower than that ordinarily achieved during the treatment of human cancers, in which successful treatment depends on the death of malignant cells. However, for the prevention of restenosis, it may be enough to inhibit activated VSMCs after injury or to prevent activation until the stimuli for growth and migration have abated.¹

Intestines of experimental animals, including 2 animals that experienced loose stools, showed no significant histopathological changes at the completion of study. These results suggest that this oral formulation of paclitaxel is relatively safe, although animals were not examined during the loose stool period; thus, further study is needed.

### TABLE 2. Angiographic and Histomorphometric Indices of Oral Paclitaxel Groups

<table>
<thead>
<tr>
<th>Paclitaxel Dose, mg/kg</th>
<th>0 (n=13)</th>
<th>5 (n=9)</th>
<th>7.5 (n=10)</th>
<th>10 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLD, AU</td>
<td>4.67±1.45</td>
<td>6.28±2.09*</td>
<td>6.97±1.79*</td>
<td>7.97±1.57*</td>
</tr>
<tr>
<td>Neointimal area, mm²</td>
<td>0.13±0.05</td>
<td>0.05±0.05*</td>
<td>0.04±0.03*</td>
<td>0.05±0.03*</td>
</tr>
<tr>
<td>Neointima/media area ratio</td>
<td>1.20±0.38</td>
<td>0.46±0.45*</td>
<td>0.39±0.29*</td>
<td>0.40±0.28*</td>
</tr>
<tr>
<td>% Area stenosis, %</td>
<td>33.9±8.6</td>
<td>15.2±15.1*</td>
<td>12.1±7.7*</td>
<td>13.5±9.7*</td>
</tr>
<tr>
<td>Media area, mm²</td>
<td>0.11±0.01</td>
<td>0.11±0.02</td>
<td>0.11±0.02</td>
<td>0.11±0.02</td>
</tr>
</tbody>
</table>

Results are reported as mean±SD. *P<0.05 vs control.
human trials have demonstrated that hematopoietic effects, the principal toxicity of paclitaxel, begin to develop only when paclitaxel plasma levels are maintained above an apparent threshold of 50 to 100 nmol/L for durations beyond (approximately) 5 hours.\textsuperscript{16,17} Five days after high-dose application, 2 of 6 animals experienced a decrease in peripheral WBC counts. These side effects could have been expected, because time above concentration of 50 nmol/L was longer than 5 hours. No side effects were discernible in the low or intermediate oral doses, although these doses were found to be as effective at inhibiting VSMC proliferation (by in situ BrdU labeling) and neointimal formation as the high oral dose.

Effective doses in animals cannot be simply extrapolated to humans, because human pharmacokinetics and metabolism may differ significantly from those of animals. Dose conversion factors for interspecies extrapolations exist.\textsuperscript{18} For example, the effective dose in human is \(\approx 1/12\) that of the mouse, \(1/6\) that of the rat, and \(1/3\) that of the rabbit in general, but it depends on the drug used. Thus, the rat dose of 5 mg/kg used in our study could be converted to a human equivalent dose of \(\approx 0.8\) mg/kg (30 mg/m\(^2\)), whereas a dose of 4.5 mg/kg (175 mg/m\(^2\)) is usually used for the treatment of human malignancy, and the development of a more efficient oral formulation could reduce this further. A combination of paclitaxel and cyclosporine is currently under phase II clinical trial in an oral form.\textsuperscript{19} This oral combination was well tolerated and did not induce gastrointestinal toxicity or myelosuppression.\textsuperscript{4}

Paclitaxel has also been investigated for the treatment of various diseases other than cancer. Phase II clinical studies investigating paclitaxel for the treatment of psoriasis and rheumatoid arthritis are currently being conducted (http://www.clinicaltrials.gov). In these studies, intravenous micellar paclitaxel (75 mg/m\(^2\) every 4 weeks) was used, which was well tolerated.

Oral everolimus (a macrolide of the same family as sirolimus) suppressed in-stent neointimal growth in the rabbit iliac artery at a dose of 1.5 mg/kg given 1 day before stenting followed by 0.75 mg/kg per day for 28 days.\textsuperscript{20} These rabbit doses could be converted to human equivalent doses of \(\approx 0.5\) and 0.25 mg/kg (3 and 1.5 mg/60 kg human), respectively. At higher doses, rabbits experienced weight loss and anorexia. Despite the efficacy of oral everolimus in animal studies, oral sirolimus did not appear to provide benefit to patients with recalcitrant restenosis, and adverse drug effects occurred frequently.\textsuperscript{21} In this study, patients were treated with the dose used in renal transplant patients (2 mg/d) for 4 weeks. Considering the high dosage and the duration of therapy, frequent adverse drug effects are not surprising. In addition, it is likely to be impossible to increase the dosage because of its systemic side effects.

The intraperitoneal dosing arm was treated as a separate experiment in the present study. To avoid daily variations, a single operator performed our experiments and similar numbers of animals were involved in control and treatment groups in a single day. Even though these 2 arms were separated, the control groups in both arms were similar by histological assessment. Angiographic evaluation (MLD, 1-point measurement) was newly introduced for supporting histological assessment and did support the histological results successfully. However, the 2 control groups were slightly different in terms of angiographic assessment. Despite this, comparisons within the same dosing arm should be reasonable, because each dosing arm has its own control.

Recently, Kolodgie et al\textsuperscript{22} reported that a single dose of systemic (intrarterial) nanoparticle paclitaxel \(\approx 2.5\) mg/kg reduced in-stent neointimal growth in the rabbit at 28 days. Blood levels of \(\approx 100\) nmol/L were achieved at a dose of 5.0 mg/kg, which was similar to that observed in the high oral paclitaxel group (108 \(\pm\) 28 nmol/L). In view of the results of systemic nanoparticle paclitaxel study in the rabbit,\textsuperscript{22} 1 or 2 doses instead of 5 may be effective and more convenient and furthermore may avoid potential side effects.

For comparison purposes, we designed the schedule of oral paclitaxel study to be similar to that of the intraperitoneal paclitaxel study.\textsuperscript{1} For this reason, day 11 after carotid injury was selected to assess neointimal formation; although this was predictive of outcome at day 14 (the typical experimental end point) in control rats in our laboratory (unpublished data), this may not be predictive of results in the longer term. In a study involving systemic paclitaxel therapy in the rabbit, the efficacy of a single dose of paclitaxel was lost at 90 days, but a repeat dose of paclitaxel given 28 days after stenting resulted in a sustained suppression of neointimal thickness at 90 days.\textsuperscript{22} Thus, further studies are needed to define ideal doses and dosing schedules (single high dose versus multiple low doses, etc), the need for booster doses in the long term, and the efficacy of combinations with localized therapies.

Cremophor EL, when administered intravenously, may produce severe hypersensitivity reactions.\textsuperscript{2} However, Cremophor EL plasma levels were undetectable in previous studies of oral paclitaxel in a Cremophor EL–containing vehicle.\textsuperscript{4} In the above studies, no hypersensitivity reactions were observed in patients.\textsuperscript{4} Although new intravenous formulations have been and continued to be developed, oral formulations should be more convenient. Because the oral delivery of paclitaxel is systemic, it should allow more uniform drug exposure to the injured arterial segment, treatment of multiple lesions, and adjustment of target dose.\textsuperscript{22}

In conclusion, we have demonstrated that oral paclitaxel provides an effective means of inhibiting proliferative response to vascular injury in the rat. Thus, oral paclitaxel could be useful for multiple lesions with or without stents. In addition, adjunctive doses could be administered to prevent late neointimal catch-up.

Acknowledgment

This study was partially funded by Hanmi Pharmaceutical Co Ltd, Korea.

References


Novel Oral Formulation of Paclitaxel Inhibits Neointimal Hyperplasia in a Rat Carotid Artery Injury Model
Dong-Woon Kim, Jin-Sook Kwon, Young-Gyu Kim, Maeng Sup Kim, Gwan-Sun Lee, Tae-Jin Youn and Myeong-Chan Cho

Circulation. 2004;109:1558-1563; originally published online March 8, 2004;
doi: 10.1161/01.CIR.0000124063.74526.BE
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/109/12/1558

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/

Downloaded from http://circ.ahajournals.org/ by guest on November 15, 2017