Age-Dependent Impairment of Reendothelialization After Arterial Injury
Role of Vascular Endothelial Growth Factor

Giuseppa Gennaro, PhD; Catherine Ménard, BS; Sophie-Élise Michaud, MS; Alain Rivard, MD

Background—The mechanisms responsible for the association between advanced age and atherosclerotic diseases are not clear. Because atherosclerosis develops in response to local endothelial injuries, we investigated the effect of aging on vascular healing and reendothelialization.

Methods and Results—Endothelium denudation was performed by balloon angioplasty of the iliac arteries in young and old New Zealand White rabbits. Planimetric analysis after Evans Blue staining at 28 days after injury showed a significant decrease in reendothelialization in old versus young animals, which was associated with an important increase in neointimal formation in old rabbits. Vascular endothelial growth factor (VEGF) was rapidly induced after balloon injury. However, arterial VEGF expression was significantly reduced in old versus young animals. To confirm the role of VEGF in the age-dependent impairment of reendothelialization, an adenoviral vector encoding for VEGF165 (adeno-VEGF) was locally delivered at the time of iliac artery angioplasty. Compared with animals treated with the control vector (adeno-βGal), reendothelialization was significantly improved and neointimal formation reduced in old rabbits treated with adeno-VEGF.

Conclusions—These results document for the first time an age-dependent impairment of reendothelialization after arterial injury. Our study indicates that VEGF supplementation may represent a useful strategy to accelerate reendothelialization and improve vascular healing in the context of aging. (Circulation. 2003;107:230-233.)

Key Words: atherosclerosis ■ aging ■ endothelium ■ growth substances

Aging is a major risk factor for atherosclerosis. It is well established that older patients present with atherosclerotic diseases that are more severe and more diffuse compared with younger patients. Similarly, older animals develop more atherosclerosis than younger counterparts with the same cholesterol levels. This suggests that aging is associated with an endogenous alteration of the vessel wall promoting atherosclerosis and vascular dysfunction.

Atherosclerotic diseases are thought to develop in response to local injuries that disrupt the protective endothelial layer. An extreme example of arterial injuries occurs during balloon angioplasty, in which the endothelial monolayer is mechanically abraded and largely removed. After such an injury, normally quiescent endothelial cells adjacent to the injured area must begin proliferating and migrating to heal the wounded area (reendothelialization). In fact, an inverse relationship has classically been observed between endothelial integrity and neointimal formation after arterial injury. Although age-dependent endothelial dysfunction has been well documented in different vascular beds, the effect of aging on arterial healing and reendothelialization after injury is currently unknown. In the present study, we tested the hypothesis that aging impairs reendothelialization.

Methods

Rabbit Iliac Artery Balloon Denudation
Young (6 to 8 months) and old (3.5 to 4 years) New Zealand White rabbits (Charles River Canada, Inc, Saint-Constant, Québec) were used for all experiments. A 4F Swan Ganz balloon catheter (Baxter) was inflated in the distal part of the iliac artery and withdrawn 3 times.

Arterial Gene Transfer
A channel balloon catheter (Boston Scientific) was used to infuse 200 μL (1 x 10^9 PFU) of a replication-defective recombinant adenovirus based on human adenovirus serotype 5 expressing human vascular endothelial growth factor (VEGF)165 under the control of the cytomegalovirus promoter (Adeno-VEGF). For controls, a human adenovirus serotype 5 expressing β-galactosidase (adeno-βGal) was used. Using this technique, we can achieve a 4% to 6% transfection rate in rabbit iliac arteries.

Evaluation of Reendothelialization
Reendothelialization was assessed by staining with Evans Blue dye (Sigma Chemical).

Evaluation of Intimal Hyperplasia
Intima/media (I/M) ratios were determined with a computerized sketching program (Clemex 3.0.032).
baseline characteristics, including weight, blood pressure, hematologic, and biochemical values, were similar in young and old animals (data not shown).

**Aging Impairs Reendothelialization and Promotes Neointimal Formation**
Planimetric analysis with Evans Blue staining at 14 days after injury (Figure 1B) reveals a trend toward reduced reendothelialization in old animals ($P=0.09$). This difference becomes highly significant at 28 days after injury (Figure 1B), revealing a 39% reduction of reendothelialization in old compared with young animals ($54.6\pm3.4\%$ versus $91.5\pm2.3\%$, $P<0.001$). The impairment of endothelial growth was associated with a significant increase in neointimal formation (Figure 1C) in old versus young animals (I/M ratio $0.95\pm0.08$ versus $0.64\pm0.05$, $P<0.01$).

**Aging Is Associated With Reduced VEGF Expression in the Arterial Wall**
Induction of VEGF by serum was significantly reduced in VSMCs isolated from the aorta of old versus young rabbits (Figure 2A). In vivo, VEGF expression was rapidly induced in iliac arteries after balloon injury. However, the ultimate level of VEGF expression in the arterial wall was significantly reduced in old compared with young animals (Figure 2B).

**VEGF Supplementation Rescues Vascular Healing in Old Animals**
The Flk1 receptor, which is responsible for most VEGF biological actions, is expressed by endothelial cells in rabbit large arteries (Figure 2C). At day 3 after angioplasty, iliac arteries of old rabbits locally transfected with adeno-VEGF showed a significant increase in VEGF expression (Figure 2D) when compared with arteries not transfected or transfected with the control vector (adeno-βGal). This is associated with a trend toward increased reendothelialization at day 14 after injury ($P=0.08$, Figure 2E), which becomes highly significant at day 28 after injury ($73.1\pm5.2\%$ versus $43.6\pm9.0\%$, $P<0.01$, Figure 2E). Moreover, treatment with adeno-VEGF leads to a significant reduction in neointimal formation in old animals (I/M ratio $0.64\pm0.02$ versus $0.77\pm0.04$, $P<0.01$, Figure 2F).

**Discussion**
These data provide the first comprehensive analysis of the effect of aging on arterial reendothelialization. In humans, impairment of endothelium-dependent vasodilation with aging (endothelial dysfunction) has been well described in different vascular beds. This "qualitative" endothelial defect has been associated with a reduction of NO availability potentially caused by oxidative stress.9 Our study suggests that aging is also associated with a “quantitative” defect in endothelial growth and vascular healing after injury. To date, aging is the only clinical phenotype found to be associated with impaired reendothelialization. Although other cardiovascular risk factors, including hypercholesterolemia and smoking, have been associated with endothelial dysfunction,5,10 previous studies have shown no detrimental effect of these risk factors.
Figure 2. Role of VEGF in the age-dependent impairment of arterial healing. A, Western blot analysis of VEGF expression after serum stimulation of VSMCs isolated from young and old rabbits. B, Western blot analysis of VEGF expression after iliac artery angioplasty in young and old rabbits. C, Immunohistochemical analysis of Flk1 expression in rabbit aorta. Negative control: primary antibody omitted. D, Western blot analysis of VEGF expression after iliac angioplasty in young and old rabbits. E, Quantification of reendothelialization at day 14 (n=4 per group) or day 28 (n=7 per group) and F, I/M ratio at day 28 (n=7 per group) after angioplasty in rabbits treated with adeno-VEGF versus adeno-βGal. *P<0.01 vs adeno-βGal.

The precise mechanisms involved in the regulation of reendothelialization are poorly understood. Here we showed that VEGF is rapidly induced in balloon-injured rabbit iliac arteries. Because VEGF-specific receptor Flk1 is expressed by endothelial cells in rabbit large arteries (Figure 2C), we hypothesized that local induction of VEGF after injury would promote the proliferation and migration of adjacent endothelial cells to heal the wounded area. We found that aging was associated with a significant decrease in VEGF expression after serum stimulation of VSMCs in vitro and after balloon angioplasty in vivo. To confirm the role of VEGF in the age-dependent impairment of reendothelialization, local VEGF supplementation was performed with an adenoviral vector. Previous studies on the efficacy of VEGF to promote reendothelialization after arterial injury have reported conflicting results.8,13 This could be because of intrinsic differences in the animal models used (rats versus rabbits) and/or because of variations in the experimental designs (protein versus plasmid administration). Moreover, it is important to note that all these previous studies were performed in young and healthy animals. Here we show that in old animals, an adenoviral-based strategy can successfully increase VEGF expression in injured arteries, accelerate reendothelialization, and reduce neointimal formation. These findings indicate that endothelial cells of old animals can still be stimulated to proliferate and migrate in response to VEGF, which is consistent with the fact that VEGF can rescue endothelial cells from senescence in vitro.14 Of note, compared with reendothelialization, the effect of VEGF supplementation on neointimal formation was found to be modest (Figures 2E and 2F). This suggests that other factors might be involved in the age-dependent increase in neointimal formation. One potential explanation is the increased proliferative activity that has been described in VSMCs isolated from old animals.15 Other mechanisms could also contribute to the impairment of reendothelialization with aging. For instance, endothelial progenitor cells have recently been shown to be involved in postnatal physiological processes.16 However, the precise role of these endothelial progenitor cells for vascular healing and whether their number and/or function are compromised during aging remain to be determined.

The findings of the present study have important clinical implications. First, age-dependent impairment of reendothelialization could contribute to explaining the increased incidence and severity of atherosclerotic diseases in older patients. Second, because of the important role of the endothelial layer on thrombogenicity and vascular tone, delayed endothelial healing after local arterial injuries could increase the incidence of acute ischemic events in the elderly. Finally, our data indicate that aging could negatively regulate endothelial recovery after angioplasty. The importance of reendothelialization in this setting was recently highlighted by clinical trials of intracoronary brachytherapy for the prevention of in-stent restenosis, in which delayed endothelial recovery was associated with a marked increase in stent thrombosis.17 The present study suggests that VEGF supplementation may represent a useful strategy to promote reendothelialization and vascular healing after angioplasty, especially in the context of aging, in which these processes are significantly compromised.

Acknowledgments

This study was supported by a grant from the Canadian Institutes of Health Research to Dr Rivard (No. 15403). Dr Rivard is a scholar from the Fédération de Recherche en Santé du Québec.

References


Age-Dependent Impairment of Reendothelialization After Arterial Injury: Role of Vascular Endothelial Growth Factor
Giuseppa Gennaro, Catherine Ménard, Sophie-Élise Michaud and Alain Rivard

Circulation. 2003;107:230-233; originally published online December 30, 2002; doi: 10.1161/01.CIR.0000050652.47145.4C

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/107/2/230

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/