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Endothelial Function in the Time of the Giants: Paul M. Vanhoutte Lecture

Donald D. Heistad

Cardiovascular Center and Departments of Internal Medicine and Pharmacology, University of Iowa
Carver College of Medicine and VA Medical Center, Iowa City, Iowa

Abstract

Paul Vanhoutte is one of the fathers of vascular biology. Among his great contributions, he demonstrated that endothelium modulates vasomotor response to vasoactive products (including serotonin) that are released when platelets aggregate in an artery. He found in arteries *ex vivo* that when endothelium is dysfunctional, in atherosclerosis or hypertension, normal relaxation to aggregation of platelets is impaired, and vessels may contract. He proposed that this mechanism may predispose to vasospasm. Our experiments *in vivo* indicated that atherosclerosis greatly potentiates vasoconstrictor responses to serotonin in the limb, brain, and eye of monkeys. We proposed that transient ischemic attacks may be mediated by platelet-induced vasospasm. We observed endothelial dysfunction in atherosclerotic monkeys, with improvement of endothelial function when hypercholesterolemia was corrected. Recently, we have studied the aortic valve (which has unique endothelium) in hypercholesterolemic mice, to examine the pathophysiology of aortic valvular stenosis. Oxidative stress is increased in stenotic valves, and severe aortic stenosis develops in about one-third of old, hypercholesterolemic mice. In stenotic aortic valves from humans, there is increased oxidative stress near calcified regions of the valves. Oxidative stress may trigger expression of pro-calcific genes in the aortic valve. Finally, we have used gene transfer of extracellular superoxide dismutase (ecSOD) to study endothelial effects of oxidative stress. Gene transfer of normal ecSOD improves endothelial dysfunction in several disease states, but gene transfer of ecSOD_{R213G}, a gene variant of ecSOD that is common in humans, fails to improve endothelial function. Gene transfer approaches may be useful to study mechanisms by which gene variants predispose to endothelial dysfunction and vascular disease.

Keywords

Endothelium; serotonin; aortic valve; oxidative stress

This is a summary of the first Paul M. Vanhoutte Lecture at the annual meeting of the American Society for Pharmacology and Experimental Therapeutics, April 2008. In the first part of this review, some of Dr. Vanhoutte's contributions will be described briefly.

In the second part of this review, I will describe some of our work. First, some vascular effects of serotonin, which are modulated by endothelium, will be summarized. Second, effects of hypercholesterolemia on the aortic valve, which has a unique endothelium, will be examined; the findings are timely, because stenosis of the valve has emerged as an extremely important clinical problem. Third, some vascular effects of one of the superoxide dismutases (SOD),

extracellular SOD (ecSOD), will be described. Vascular effects of a common human gene variant will be described, and a novel way to study gene variation will be illustrated.

Paul M. Vanhoutte

Dr. Vanhoutte is one of the fathers of vascular biology. He is a pioneer in what might be called “translational endothelial biology.” When Robert Furchgott discovered endothelium-derived relaxing factor (EDRF) (1), Vanhoutte examined endothelial function in several disease states and thereby translated Furchgott’s findings into pathophysiology, as well as physiology.

Paul Michel Vanhoutte was born in Belgium, and received a Doctor of Medicine, Surgery, and Obstetrics (equivalent of an M.D.) in Ghent, Belgium. He worked with Drs. Bouckaert and Leussen in Ghent, and received an Aggregation in Higher Education (equivalent of a Ph.D.) in Antwerp. He worked with Dr. John Shepherd in Rochester, Minnesota, and then worked at the University of Antwerp, the Mayo Clinic, Baylor College of Medicine, and Servier Research Institute in Paris. Vanhoutte now is Director of the Biopharmaceutical Development Center and Head of the Department of Pharmacology at the University of Hong Kong.

Vanhoutte organizes “Mechanisms of Vasodilatation” meetings, which bring together scientists who study vascular biology. When Furchgott and Zawadzki published abstracts in *The Pharmacologist* and *Federation Proceedings* that described release of EDRF by acetylcholine in 1979 and 1980, Vanhoutte invited Furchgott to present his work at a meeting in Wilrijk, Belgium -- “Mechanisms of Vasodilation II.” When Furchgott described his finding, I did not appreciate the importance of the work. I thought that, because acetylcholine does not circulate in the blood, it was unlikely to reach the endothelium and release EDRF. Vanhoutte, Salvador Moncada, and other giants in endothelial biology were not as narrow in their thinking. They quickly demonstrated that endothelium releases EDRF in response to a wide variety of stimuli, including activated platelets and products released by platelets (including adenosine diphosphate (ADP) and serotonin) (2–4).

When talking about endothelial function, Vanhoutte starts his talks at “Mechanisms of Vasodilatation” and other meetings by acknowledging Furchgott’s primacy in this area of research. I was, and am, impressed by Vanhoutte’s faithfulness in attribution of discoveries to appropriate scientists.

Vanhoutte’s ability to organize great meetings influenced the decision of the Nobel Committee in 1998. In 1986, at “Mechanisms of Vasodilatation V” in Rochester, Minnesota, Furchgott and Ignarro presented evidence that nitric oxide is EDRF. That meeting proved to be crucial, as acknowledged by Professor Sten Lindahl, when Furchgott, Ignarro, and Murad received the Nobel Prize for “Nitric Oxide as a Signaling Molecule in the Cardiovascular System” in 1998 (Fig. 1).

Paul Vanhoutte is an amazingly prolific investigator in endothelial biology, and other areas of vascular biology. He is a truly extraordinary mentor, and has trained over 100 fellows (Table 1). Vanhoutte’s trainees, several of whom are also international leaders in cardiovascular research, continue to seek his advice as an “academic father.”

Vascular Effects of Serotonin

The remainder of this review will focus on some of our work. I apologize to other authors about failing to refer to their work, because of limitations in number of references.

Because of Henry and Yokoyama’s finding (5) that vasoconstrictor responses to serotonin are augmented in atherosclerotic vessels *ex vivo*, we tested the hypothesis that vasoconstrictor

responses to serotonin in vivo are exaggerated by atherosclerosis. We found that atherosclerosis produces enormous potentiation of vasoconstrictor responses to serotonin in the large arteries of hind limb of monkeys (6). We also found that serotonin, which produces minimal constriction of retinal arteries in monkeys, produces choroidal and retinal vasospasm in atherosclerotic monkeys (Fig. 2) (7) and a profound reduction in ocular blood flow, accompanied by transient flattening of the electroretinogram (8). Thus, we suggested that transient ischemic attacks (TIAs), which are characterized by both transient cerebral ischemia and transient blindness, may be mediated by vasospasm initiated by release of serotonin from platelets (7,8).

What is the mechanism of hyperresponsiveness to serotonin in atherosclerotic vessels? Vanhoutte and his colleagues had shown that vascular responses to serotonin are modulated by endothelium (3,4). They found that, when endothelium is normal, serotonin releases EDRF (nitric oxide) and produces vascular relaxation. In contrast, Vanhoutte found that, when endothelium is removed or dysfunctional, serotonin contracts blood vessels. We wondered whether potentiation of vasoconstrictor responses to serotonin in atherosclerotic arteries might be mediated by impairment of endothelial function. This question led us to examine endothelial function in atherosclerotic arteries, and the finding that atherosclerosis profoundly impairs endothelial function (9). I should acknowledge that the hyperresponsiveness to serotonin that we observed in the limb, retinal vessels, and other vascular beds turned out to be mediated by other mechanisms, in addition to endothelial dysfunction.

Why is hyperresponsiveness to serotonin important in atherosclerosis? Aggregation of platelets releases several vasoactive substances, including serotonin, ADP, and thromboxane. Vanhoutte showed that endothelium modulates vascular responses to activation of platelets (2), so that relaxation to activation of platelets ex vivo is impaired or even changes to contraction when endothelium is removed. Importantly, he also showed that, in the presence of several disease states (hypertension, diabetes, etc.) vascular responses to activation of platelets are altered profoundly.

Because of Vanhoutte's findings in blood vessels ex vivo, Dr. Sanjay Kaul examined vascular responses to activation of platelets in vivo. We found that vasodilator responses to activation of platelets are profoundly impaired by atherosclerosis (10–12). This led us, and others (including Vanhoutte (3) and Willerson (13)), to speculate that endothelial dysfunction may contribute to susceptibility of atherosclerotic vessels to vasospasm.

Many of our experiments, and those of others, focused on the role of endothelium in modulation of vasomotor responses in large (conduit) arteries. We then initiated experiments to examine endothelial function in small (resistance) vessels, by studying modulation of resistance in monkeys. Responses to acetylcholine were examined in the perfused hindlimb of monkeys. A nitric oxide synthase (NOS) inhibitor was given to demonstrate (presumably) that reduction in resistance in response to acetylcholine is blocked by NG-monomethyl-L-arginine (L-NMMA), and thus mediated by NOS. To our great surprise, we could not attenuate vasodilator responses to acetylcholine by administration of L-NMMA in two monkeys (unpublished data).

We then pursued this mechanism in the hindlimb of normal rabbits, and arrived at the same conclusion (14). Although NOS inhibitors increased basal resistance (which is mediated by NO) in the limb, NOS inhibitors did not attenuate dilator responses of resistance vessels to acetylcholine. We did not pursue the mechanism further, but assume that an endothelium-derived hyperpolarizing factor (EDHF), as extensively studied by Vanhoutte (15) and several other investigators, contributes importantly to dilator responses of resistance vessels to acetylcholine. If, in some vascular beds, EDHF (or several EDHFs) mediate decreases in vascular resistance to endothelium-dependent agonists, EDHFs are of great importance in

physiology and pathophysiology. EDHF is now a major focus of “Mechanisms of Vasodilatation” meetings which Vanhoutte organizes.

We have suggested that there is a stereotypical pattern of vascular changes in atherosclerosis and hypertension. We proposed that there is a “sick vessel syndrome” (16). The hallmarks of this syndrome are: endothelial dysfunction, relative preservation of relaxation of vascular muscle, exaggerated vasoconstrictor responses (especially to serotonin), and structural changes that are generally adaptive and protective. In our experience, with a variety of disease states, hyperresponsiveness to serotonin (which typically is far greater in magnitude than endothelial dysfunction) is the most consistent marker of vascular disease.

A key question, in relation to potential benefits of treatment of hypercholesterolemia, is whether atherosclerotic lesions can regress, with reabsorption of lipid from arteries and improvement of endothelial function. The question was answered many years ago, first in monkeys (17–20) and then in patients (21,22). A surprising finding was that, in contrast to an enormous reduction in myocardial infarction and stroke during reduction of plasma cholesterol, structural changes of arteries (as measured by luminal diameter or intimal/medial area) improve only minimally (22). The explanation for this paradox is that, as lipid and inflammatory cells leave the atherosclerotic artery, collagen increases in the artery, so that intimal/medial area is reduced only slightly, and increases in luminal diameter are limited. The vulnerable plaque becomes more stable (and less likely to rupture and produce arterial thrombosis), however, as arterial lipid and inflammation decrease and collagen increases (23).

Although structural improvement of arteries (as measured by intimal/medial area) is modest during regression of atherosclerosis, there is striking improvement in function in the arteries. We found in monkeys that endothelial function improves rapidly during treatment of hypercholesterolemia (18). The finding was confirmed in humans (21). In addition, hyperresponsiveness to serotonin subsides very rapidly during treatment of hypercholesterolemia and regression of atherosclerosis (24).

Generation of superoxide in the arterial wall, with inactivation of nitric oxide, contributes importantly to endothelial dysfunction in atherosclerosis. Furthermore, improvement of endothelial function during regression of atherosclerosis is accompanied by (and presumably mediated by) reduction of vascular superoxide (25). These findings support the concept that oxidative stress plays a key role in the pathophysiology of atherosclerosis.

Aortic Valvular Stenosis

Endothelium of the aortic and other cardiac valves is subjected to unusual flow dynamics. Blood is ejected rapidly past endothelium on the ventricular side of the valve. When the valve closes during diastole, endothelium on the aortic side of the valve is exposed to turbulent (or disordered) blood flow. A striking finding is that endothelium of the aortic valve maintains a unique phenotype in vitro (26). During flow in vitro, aortic endothelium aligns in the direction of flow, but aortic valvular endothelium aligns perpendicular to flow (Fig. 3).

The pathophysiology of aortic valvular stenosis is of interest because it is an enormously important clinical problem, and because risk factors and histopathology suggest a close relationship to atherosclerosis (27). Risk factors include older age, hypercholesterolemia, male sex, smoking, and hypertension. Stenotic aortic valves resemble atherosclerotic lesions, with calcification, lipid deposition, inflammation, and matrix remodeling.

We stumbled upon the first experimental model of aortic valvular stenosis, when we obtained “Reversa” mice from Dr. Stephen Young (28). The mice are lipoprotein receptor deficient ($LDLR^{-/-}$) and apolipoprotein B-100 only ($ApoB^{100/100}$), which increases susceptibility to

atherosclerosis. When some mice became too old to study as part of a protocol to examine endothelial function, we chose to determine whether superoxide is increased in the aortic valve. The rationale was that reactive oxygen species (ROS) are increased in atherosclerosis, and ROS might also be increased in the aortic valve of atherosclerotic mice. We found a striking increase in superoxide in the aortic valve of LDLR^{-/-} ApoB^{100/100} mice (Fig. 4).

Dr. Robert Weiss then used echocardiographic imaging and microtransducer-tipped catheters to determine whether there was evidence of aortic valvular stenosis in these mice (29). About one-third of 20-month old LDLR^{-/-} ApoB^{100/100} mice had severe aortic stenosis, with an estimated 75% reduction in valve area, left ventricular hypertrophy, and a 35 to 70 mm Hg peak pressure gradient across the aortic valve. We were delighted to have discovered the first experimental animals with spontaneous development of the full syndrome of aortic valvular heart disease.

We have pursued these findings in humans. Dr. Jordan Miller tested the hypothesis that oxidative stress is increased in calcified aortic valves in humans, and examined mechanisms that increase or protect against oxidative stress in the valves (30). He found that, in normal aortic valves (from human hearts that were not suitable for transplantation), superoxide levels are low and homogenous throughout the valve. In stenotic valves (removed during surgical replacement of the valve), superoxide was normal in non-calcified regions and increased two-fold near calcified regions of the valve. Thus, oxidative stress is increased in calcified regions of stenotic aortic valves. Dr. Francisco Laurindo also has found oxidative stress in aortic valves, obtained from humans at surgery or autopsy (31).

Mechanisms responsible for oxidative stress in the aortic valve were a surprise to us, because they differ from mechanisms observed previously in atherosclerotic arteries. In atherosclerotic plaques, upregulation of NAD(P)H oxidase plays a key role in increasing oxidative stress, and expression of antioxidant enzymes (including SODs) is generally unchanged or increased (32). In contrast, in stenotic aortic valves from humans, we found that NAD(P)H oxidase activity is not increased, nitric oxide synthase may be “uncoupled,” and SOD activity is decreased. In addition, levels of hydrogen peroxide are markedly elevated in calcified regions of stenotic valves, and catalase expression is reduced (30). Thus, there appear to be fundamental differences in mechanisms that account for increased oxidative stress in atherosclerotic arteries and in stenotic aortic valves.

A key question is whether increases in oxidative stress contribute to development of valvular calcification, or whether oxidative stress is a consequence of valvular stenosis. The answer to this question is not known, but there are many proteins and transcription factors in blood vessels that are redox-sensitive (Table 2). Thus, we are intrigued by the possibility that increases in reactive oxygen species in the aortic valve may play an important role in the pathophysiology of aortic valvular calcification, by initiating signaling of procalcific pathways.

If pharmacological approaches can be demonstrated to slow or stop the progression of aortic valvular stenosis, one might avoid surgical replacement of the valve. Retrospective studies suggest that HMGCoA-reductase inhibitors (“statins”) and angiotensin converting enzyme inhibitors may slow the progression of stenosis of the aortic valve. Prospective studies of angiotensin-converting enzyme inhibitors have failed to show slowing of progression of stenosis, and studies of statins have provided conflicting results (33,34). Results of randomized trials are not yet available. We are concerned that, in ongoing randomized trials, statins are being used in patients with moderately severe stenosis. We speculate that statins may not be effective at this advanced stage of stenosis, and may be much more effective in slowing progression of aortic stenosis when given very early in the process.

“Reversa” mice have a conditional allele of the gene encoding for microsomal triglyceride transfer protein (MTTP) and inducible Mx1-Cre transgene (28). Thus after induction of Cre expression with polyinosinic-polycytidylic acid, the genetic “switch” deletes the MTTP gene, and hypercholesterolemia in the mice decreases quickly to normal levels. Our preliminary evidence suggests that reduction of hypercholesterolemia at an early stage may result in removal of lipid from the aortic valve and abrogate the development of aortic valvular stenosis (35).

Vascular Effects of an SOD Gene Variant

Oxidative stress, and antioxidant mechanisms, are of great interest in vascular biology. We are especially interested in the role of extracellular SOD (ecSOD), because ecSOD is highly expressed in blood vessels, protects against inactivation of nitric oxide (36), and thereby helps to preserve endothelial function.

Several years ago, Dr. Yi Chu demonstrated that gene transfer of ecSOD improves endothelial function in spontaneously hypertensive mice (SHR) and reduces arterial pressure (37). Of particular interest was the finding that beneficial effects of ecSOD require the heparin-binding domain (HBD), which mediates binding of ecSOD to heparan sulfate proteoglycans on cell surfaces.

There is a gene variant, R213G, in the HBD of ecSOD which is common (about 2%–5% of the population) in humans. A large association study demonstrated increased risk of ischemic heart disease in heterozygotes carrying ecSOD_{R213G} (38). The ecSOD_{R213G} gene variant does not reduce enzymatic activity of ecSOD, but binding to endothelial cells is impaired. Thus, one might predict that the ecSOD_{R213G} gene variant would lead to increased susceptibility to oxidative stress and endothelial dysfunction.

Yi Chu constructed an adenovirus that expresses ecSOD_{R213G} to examine vascular effects of the gene variant (39). Studies of gene variants are of great interest, as *Science* described “Human Genetic Variation” as the “Breakthrough of the Year” for 2007. In addition to studies to determine *which* gene variants cause human disease, a major goal is to determine *how* gene variants produce disease. We have developed a method, using adenoviral gene transfer, to study a gene variant (or “mutant”). This method complements studies of human phenotype of gene variants, and studies of “knock-out” and “knock-in” transgenic mice.

We examined vascular effects of ecSOD_{R213G} and normal ecSOD. Our goal was to determine whether gene transfer of ecSOD_{R213G} has less beneficial effect than normal ecSOD on vasomotor function and arterial pressure in SHR. Recombinant adenoviruses were injected intravenously. Adenoviruses are removed in the liver, and we anticipated that, after infection of hepatocytes, ecSOD_{R213G} and normal ecSOD proteins would be produced in the liver, released into the circulation, and bound by endothelial cells.

We found that, as in a previous study (37), gene transfer of normal ecSOD reduces arterial pressure and superoxide in the aorta, and improves endothelial function in SHR. Binding to endothelial cells and blood vessels was much less by ecSOD_{R213G} than by normal ecSOD. The novel finding was that ecSOD_{R213G}, in contrast to normal ecSOD, has no significant protective effect on arterial pressure, vasomotor function, or vascular superoxide in SHR (Fig. 5). Its binding to endothelial cells (in vessels) was greatly impaired (39).

We also have observed that normal ecSOD, but not ecSOD_{R213G}, protects against endothelial dysfunction in rats with heart failure (40), and in mice with inflammation produced by bacterial endotoxin (41). The findings provide a possible mechanism by which humans with ecSOD_{R213G} are predisposed to vascular disease.

Closing

In addition to being a great scientist and mentor, Paul Vanhoutte is a wonderful friend to many people. His studies of blood vessels *ex vivo* have inspired many of our studies. It was a great honor and privilege to give the first Paul M. Vanhoutte Lecture in 2008.

I have been fortunate to have many scientists work in my laboratory during the past 30 years. The opportunity to work with these young people, and to get to know them and their families, is a great joy and one of the most satisfying experiences of my life.

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“...The hunt for Furchgott’s endothelial factor came to an end during a scientific meeting at the Mayo Clinic...in the summer of 1986. At the meeting Furchgott concluded...that the factor was identical with NO. Ignarro supported this at the same meeting...[and] went one step further.... The hunt was over. The riddle concerning the endothelial factor was finally solved....”

Figure 1.

Presentation speech by Professor Lindahl at award of the Nobel Prize in Physiology or Medicine, Stockholm, 1998.

Figure 2. Fluorescein angiograms in two atherosclerotic monkeys, under control conditions (left panels) and during infusion of serotonin i.v. (right panels). In one monkey, serotonin produced spasm of the two choroidal arteries, and abolished blood flow to the choroid (upper right panel). In another monkey, serotonin produced spasm of the retinal artery and one choroidal artery (lower right panel). From Hayreh et al (7).

Figure 3.

Alignment of porcine aortic endothelial cells (left) and porcine aortic valve endothelial cells (right) during flow in vitro. Aortic cells align parallel to flow, and aortic valvular cells align perpendicular to flow. From Butcher and Nerem (26).

C57 ***LDLr^{-/-}ApoB^{100/100}***

Figure 4. Dihydroethidium fluorescence in aortic valve and aorta from a normal mouse (left) and a hypercholesterolemic mouse with aortic stenosis (right). Increased fluorescence indicates an increase in superoxide. From Weiss et al (29).

Figure 5.

Effects of gene transfer of $ecSOD_{R213G}$ in spontaneous hypertensive rats (SHR) on superoxide in aorta (left panel), systolic pressure (middle panel), and relaxation of carotid artery to acetylcholine (right panel). Values are compared to normotensive Wistar-Kyoto (WKY) rats.

* = $p < 0.05$ vs. Control. Adapted from Chu et al (39).

Table 1

Fellows of Paul Vanhoutte

Auch-Schwelk, W. (Germany)	Ge, T. (PR China)	Nonotte, I. (France)
Biondi, M.L. (Italy)	Gisclard, V. (France)	Olmos, L. (Venezuela)
Blaise, G. (Belgium)	Gluais, P. (France)	O'Rourke, S.T. (USA)
Boels, P.J. (Belgium)	Gräser, T. (Germany)	Park, S.J. (USA)
Bond, R.A. (USA)	Guo, H. G. (PR China)	Pearson, P.J. (USA)
Borg-Capra, C. (France)	Harker, C.T. (USA)	Perrault, L.P. (Canada)
Bos, R. (France)	Hoeffner, U. (Germany)	Quignard, J.F. (France)
Boulanger, C. (France)	Hoshino, Y. (Japan)	Rasmussen, L.E. (Denmark)
Brouri, F. (Algeria)	Houston, D.S. (USA)	Rimele, T.J. (USA)
Brum, J.M. (Brazil)	Illiano, S.C. (France)	Rooke, T. (USA)
Busk, M.F. (USA)	Iqbal, A. (Pakistan)	Rubanyi, G. (Hungary)
Bychlov, R. (Russia)	Janczewski, P. (Poland)	Rusch, N.J. (USA)
Castedo-Delrieu, M. (France)	Janssens, W.J. (Belgium)	Sanchez-Ferrer, C. F. (Spain)
Chataigneau, T. (France)	Joly, G.A. (France)	Sansilvestri-Morel, P. (France)
Coen, E. (Belgium)	Junquero, D.C. (France)	Schini, V.B. (France)
Cohen, R.A. (USA)	Katusic, Z.S. (Yugoslavia)	Scott-Burden, T. (Zimbabwe)
Collis, M.G. (UK)	Kennedy, S. (UK)	Shibano, T. (Japan)
Cooke, J.P. (USA)	Kerth, P.A. (Sweden)	Shimokawa, H. (Japan)
Corriu, C. (France)	Kim, N.D. (South Korea)	Soares-De-Moura, R. (Brazil)
Dalemans, P. (Belgium)	Kim, P. (South Korea)	Stuart-Smith, K. (UK)
Danser, A.H.J. (Netherlands)	Komori, K. (Japan)	Taddei, S. (Italy)
Daskalopoulos, D.A. (Greece)	Lee, M.Y.K. (Hong Kong)	Tang, E.H.C. (Hong Kong)
Day, N.S. (USA)	Lindblad, L.E. (Sweden)	Thollon, C. (France)
De Ceuninck, F. (France)	Lüscher, T.F. (Switzerland)	Tuncer, M. (Turkey)
De Mey, C. (Belgium)	Marsault, R. (France)	Uhrenholt, T.R. (Denmark)
De Mey, J.G. (Belgium)	McGoon, M.D. (USA)	Van Hee, R.H. (Belgium)
De Vriese, A.S. (Belgium)	Miao, C.Y. (PR China)	Verbeuren, T.J. (Belgium)
Desta, B. (Ethiopia)	Michel, F. (France)	Vercruysse, P. (Belgium)
Driessens, M. (Belgium)	Miller, V.M. (USA)	Vedernikov, Y.P. (Russia)
Duprez, D.A. (Belgium)	Mombouli, J-V. (Congo)	Vidal, M.J. (France)
Durante, W. (USA)	Brazzaville)	Webb, R.C. (USA)
Eber, B. (Austria)	Morrison, K.J. (UK)	Weigert, A.L. (Brazil)
Edoute, Y. (Israël)	Muldoon, S.M. (Ireland)	Yang, D. (PR China)
Félétou, M. (France)	Nagao, T. (Japan)	Zander, J.F. (Germany)
Flavahan, N.A. (UK)	Nakashima, M. (Japan)	Zellers, T.M. (USA)
Gao, Y. (PR China)		

TABLE 2

Some proteins/transcription factors in vascular tissue that are positively regulated by oxidative stress:

Osteocalcin	CBFA1
Bone sialoprotein	BMP-7/Osteogenic protein-1
Osteopontin	BMP-2
RANK-ligand	Matrix gla protein
Osteonectin	