Toward functional genomics of flow-induced outward remodeling of resistance arteries

Jo G. R. De Mey, Paul M. Schiﬀers, Rob H. P. Hilgers, and Marijke M. W. Sanders

Department of Pharmacology and Toxicology, Cardiovascular Research Institute Maastricht, University of Maastricht, and School of Life Sciences, Transnational University of Limburg, Maastricht, The Netherlands

Submitted 6 August 2004; accepted in ﬁnal form 26 September 2004

De Mey, Jo G. R., Paul M. Schiﬀers, Rob H. P. Hilgers, and Marijke M. W. Sanders. Toward functional genomics of ﬂow-induced outward remodeling of resistance arteries. Am J Physiol Heart Circ Physiol 288: H1022–H1027, 2005; doi:10.1152/ajpheart.00800.2004.—In resistance-sized arteries, a chronic increase in blood ﬂow leads to increases in arterial structural luminal diameter and arterial wall mass. In this review, we summarize recent evidence that outward remodeling of resistance arteries 1) can help maintain and restore tissue perfusion, 2) is not intimately related to ﬂow-induced vasodilatation, 3) involves transient dedifferentiation and turnover of arterial smooth muscle cells, and 4) is preceded by increased expression of matricellular proteins, which have been shown to promote disassembly of focal adhesion sites. Studies of experimental and physiological resistance artery remodeling involving differential gene expression analyses and the use of knockout and transgenic mouse models can help unravel the mechanisms of outward remodeling.

Flow-induced vasodilatation; matricellular protein; gene expression microarray analysis

In arteries, transmural pressure and blood ﬂow inﬂuence wall thickness and structural luminal diameter (13, 16, 28, 29, 49). Mathematical modeling, cell and molecular biological approaches, and experiments in genetically modiﬁed animals are beginning to shed light on the mechanisms that underlie these arterial remodeling responses. Here, we summarize recent observations concerning ﬂow-induced outward remodeling of resistance arteries. Exploration of this type of arterial structural response might lead to pharmacological treatments to 1) improve collateral perfusion and 2) reverse the inward arterial remodeling in essential hypertension.

Terminology

Remodeling is frequently used to describe any alteration of arterial structure. In agreement with others (4, 38), we ﬁnd it useful to note the differences among several types of arterial remodeling: 1) neointima formation, 2) alteration of the arterial structural luminal diameter (outward or inward), and 3) alteration of tunica media mass (hypertrophic, eutrophic, and hypotrophic). Although this classiﬁcation is helpful, it also highlights gaps in current knowledge, such as the manner in which media, adventitia, and ultrastructural wall components, such as extracellular matrix, focal adhesion sites, and cytoskeleton, determine the diameter. Arterial structural diameter refers to the maximally dilated diameter and results from the elastic properties of the arterial wall. The diameter of an arterial segment in vivo obviously depends on this structural diameter and on the degree of vasomotor tone or smooth muscle contractile activity.

Resistance arteries are small (300–100 μm diameter) muscular arteries that are situated between large elastic conduit arteries and arterioles. In contrast to the large arteries, resistance arteries 1) not only respond to, but also inﬂuence, local mean arterial pressure and blood ﬂow, 2) rarely, if ever, develop a neointima, and 3) do not display hypertrophy but, rather, exhibit inward eutrophic remodeling during chronic hypertension (37). In resistance arteries, circumferential wall stress and wall shear stress are maintained at higher values than in arterioles (43), and the number of feeding resistance arteries in a vascular bed is less susceptible to chronic modulation than the number of arterioles and capillaries (42). An exception is the upgrading of preexisting collateral arterioles into resistance arteries after occlusion of a major feed artery. This arteriogenesis (20) represents outward hypertrophic remodeling.

Driving Forces of Remodeling

Anatomic analyses and experimental animal studies suggest that wall shear stress and circumferential wall stress are maintained constant throughout the systemic arterial tree (16, 28), with the exception of the true microcirculation, where these parameters change progressively toward venous values (43). Angioadaptation, representing arterial responses to the interacting inﬂuences of altered wall shear stress and circumferential wall stress (43), can be brought about acutely by altered vasomotor tone and chronically by structural autoregulation or arterial remodeling. Circumferential wall stress and wall shear stress are considered major driving forces during morphogenesis and remodeling of arteries. They do, however, not suﬃce to predict the architecture of arterial beds or the existence of arcading collateral arteries, which are abundant in vascular beds that undergo marked inﬂections in blood ﬂow (e.g., the female urogenital system and the gastrointestinal tract). Therefore, additional inﬂuences, such as metabolic

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
signals conducted upstream along the arterial tree (for review see Ref. 43), must be considered. The relation between arterial vaso-
motor and structural responses to stress changes is the subject of
intense research.

FLOW-INDUCED VASODILATATION AND FLOW-INDUCED
REMODELING OF RESISTANCE ARTERIES

A chronic increase in blood flow has been reported by
several groups to result in outward hypertrophic remodeling of
resistance arteries (8, 9, 41, 52, 54, 55, 57). A widely used
experimental animal model involves ligation of feed arteries in
resistance arteries (8, 9, 41, 52, 54, 55, 57). A widely used

...
the large number of unknowns, curiosity-driven research may help build testable hypotheses about the mechanisms of outward remodeling.

STRUCTURAL CHANGES DURING FLOW-INDUCED REMODELING

In the rodent mesenteric artery model of flow-induced outward resistance artery remodeling, signs of SMC turnover and dedifferentiation have been reported. In arteries exposed in vivo to twice the normal blood flow, increased bromodeoxyuridine labeling, increased immunoreactivity for proliferating cell nuclear antigen, and a significant increase in arterial SMC number indicate stimulated arterial SMC proliferation (8, 54). Signs of arterial SMC apoptosis, such as DNA laddering and increased TdT-mediated dUTP nick end labeling, were equally evident in chronically hyperperfused arteries (twice normal blood flow) undergoing outward hypertrophic remodeling and in chronically hypoperfused arteries (0.1 times normal blood flow) undergoing hypotrophic inward remodeling (8). Apoptosis and proliferation of SMC in different layers of the media may help the wall expand, but this remains to be established. Along with increased arterial SMC turnover, a drop in the immunoreactivity of the arterial wall for desmin and caldesmon was documented, indicating (transient) dedifferentiation of arterial SMC (8). Dedifferentiated synthetic arterial SMC are more prone to proliferate, migrate, and synthesize and degrade their matrix (4, 40). Matrix alterations during resistance artery remodeling have remained elusive, partly because of the low density of this material in the wall. Work in large arteries, such as the mouse common carotid artery, strongly suggests that marked flow-related inward or outward arterial remodeling can proceed without significant modifications in the arterial collagen and elastin contents (23). It may be hoped that vital molecular imaging of arterial wall structure will reveal the ultrastructural basis of the arterial structural luminal diameter. Cells, nuclei, collagen, and elastin fibers can easily be visualized, and other matrix components such as fibronectin can be traced with the appropriate antibodies. Multiphoton lifetime laser scanning confocal microscopy (33, 59) has the necessary penetration and spatial resolution to approach these issues in intact resistance arteries in relation to transmural pressure and blood flow.

Table 1. Flow-induced vasodilatation and flow-induced outward remodeling in rodent experimental animal models

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Model</th>
<th>Species</th>
<th>Artery</th>
<th>FID</th>
<th>FIR</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desmin&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Ligation</td>
<td>Mouse</td>
<td>Mesenteric</td>
<td>Reduced</td>
<td>Normal</td>
<td>32</td>
</tr>
<tr>
<td>Dystrophin&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Ligation</td>
<td>Mouse</td>
<td>Mesenteric</td>
<td>Reduced</td>
<td>Reduced</td>
<td>31</td>
</tr>
<tr>
<td>Vimentin&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>None</td>
<td>Mouse</td>
<td>Mesenteric</td>
<td>Reduced</td>
<td>ND</td>
<td>21</td>
</tr>
<tr>
<td>TissueACE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Pregnancy</td>
<td>Mouse</td>
<td>Carotid</td>
<td>Increased</td>
<td>Normal</td>
<td>47</td>
</tr>
<tr>
<td>t-Kallikrein&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Ligation</td>
<td>Mouse</td>
<td>Carotid</td>
<td>Reduced</td>
<td>Normal</td>
<td>21</td>
</tr>
<tr>
<td>eNOS&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Ligation</td>
<td>Mouse</td>
<td>Carotid</td>
<td>Reduced</td>
<td>Normal</td>
<td>45</td>
</tr>
<tr>
<td>L-NAME</td>
<td>Ligation</td>
<td>Rat</td>
<td>Mesenteric</td>
<td>ND</td>
<td>Normal</td>
<td>9</td>
</tr>
<tr>
<td>Aging</td>
<td>Ligation</td>
<td>Rat</td>
<td>Mesenteric</td>
<td>Normal</td>
<td>Reduced</td>
<td>53</td>
</tr>
<tr>
<td>Aging</td>
<td>Pregnancy</td>
<td>Mouse</td>
<td>Uterine</td>
<td>Normal</td>
<td>Reduced</td>
<td>34</td>
</tr>
</tbody>
</table>

ACE, angiotensin-converting enzyme; L-NAME, chronic treatment with L<sup>−</sup>-nitro-L-arginine methyl ester; ND, not determined; FID, flow-induced vasodilatation; FIR, flow-induced outward remodeling; eNOS, endothelial nitric oxide synthase; t-kallikrein, tissue kallikrein.

The small size of resistance arteries, which might be beneficial for imaging purposes, complicates molecular biological analyses during remodeling. However, with techniques that are standard practice in single cell studies, a sufficiently large mRNA pool can be generated from isolated resistance arteries. Recently, using DNA microarrays after T7 RNA polymerase amplification of the extracted mRNA, we compared gene expression in rat mesenteric resistance arteries exposed for 1–32 days in vivo to normal or twice the normal blood flow in vivo (57). For a large fraction of the genes (>800 of 10,500), the expression was modified by more than twofold during exposure to altered blood flow in vivo. These included 1) several growth factors (receptors), oncogenes, cell cycle progression factors, and modulators of apoptosis, consistent with the above-mentioned increased turnover of arterial SMC, and 2) many cytoskeletal and contractile protein markers of arterial SMC phenotype, consistent with the above-mentioned dedifferentiation. These alterations reached statistical significance after a few days, suggesting that they were involved in the execution, but not in the initiation, of the outward hypertrophy. The number of genes from which the expression was rapidly modified (<24 h) was, however, comparatively small (44 of 10,500, 18 of which were downregulated and 26 upregulated). They included a downregulation of the 82-kDa, but not the 70-kDa, subunit of soluble guanylyl cyclase, which is compatible with chronic activation of the NO-guanylyl cyclase-cGMP-protein kinase G pathway (7). They also included transient upregulation of the expressions of thrombospondin (TSP)-1, but not TSP-2 or TSP-3; tenascin C (TNC), a desintegrin and metalloproteinase with TSP motifs (ADAMTS-1), and membrane type 2 metalloproteinase (MMP)-15, but not MMP-2, MMP-7, or MMP-9 (Fig. 2). In view of the recent literature on matricellular proteins (10, 26, 30, 39), these findings are compatible with the working hypothesis outlined in Fig. 3. TSP-1 stimulates activity of MMP-2, which produces fibrillar collagen, which in turn promotes TNC production. TSP-1 and TNC promote disassembly of focal adhesion sites through mechanisms that require protein kinase G and inhibition of the RhoA-Rho kinase pathway. Subsequently, arterial SMC can slide, dedifferentiate, and proliferate more easily. This hypothesis is compatible with the temporal changes in
gene expression, dedifferentiation, proliferation, and outward remodeling and with the observation that although pharmacological inhibition of Rho kinase modifies inward remodeling, it does not alter outward remodeling (57). Dedicated drug-intervention, transgenic, knockout, and rescue studies are required to strengthen this hypothesis. Because only a few pharmacological tools modulate the presence and activity of matricellular proteins, transfection techniques (6) may be considered for these purposes.

**PHYSIOLOGICAL FLOW-INDUCED RESISTANCE ARTERY REMODELING**

The mesenteric artery ligation model that was frequently used in rats can also be applied, to some extent, to mice. Knockout mice can provide evidence for the involvement (or noninvolvement) of key molecules in flow-induced arterial remodeling (31, 32). Transgenic expression of reporter constructs has recently been applied to visualize effects of blood flow on local gene expression in vivo (48). Other more indirect approaches may be considered to benefit from the growing collection of mouse models.

**Development.** During growth and development, local blood flow and arterial size increase. This setting might be instrumental in investigation of the mechanisms whereby tissue size and activity, local blood flow, and arterial size are matched to each other. Two extreme models illustrate the potential of this approach. In growth hormone transgenic mice (12), body weight (>50 g), peripheral organ weight, and resistance artery size are proportionally increased compared with wild-type mice (35 g body wt). The size of the brain and of the carotid arteries is, however, not modified in this model. In mice that lack a hypoxia-responsive element in the promoter sequence of their vascular endothelial growth factor (VEGF) genes (27), blunt angiogenesis probably results in severe growth retardation (<20 g) and small hearts, kidneys, skeletal muscles, and gastrointestinal tract, while the brain is relatively spared (15). In these VEGF−/− mice, the luminal diameter and media mass are reduced in mesenteric and saphenous arteries, but not in carotid arteries (unpublished observations).

**Pregnancy.** During mammalian pregnancy, uterine blood flow increases from <1% of cardiac output to >20% of the 1.3-fold increased cardiac output. Angiogenesis, estrogen-induced upregulation of endothelial NOS, vasodilation, and remodeling of spiral arteries and uterine arteries contribute to the increased blood flow to the growing uterus and fetuses (5). The luminal diameter and media mass of uterine arteries increase markedly in pregnant mice (22, 23). This increase is accompanied by dedifferentiation of the SMC (19). After pregnancy, most of the uterine arterial structural changes are rapidly reversed (23). Maternal deficiency in key modulators of endothelium-dependent flow-induced vasodilatation, such as tissue angiotensin-converting enzyme, tissue kallikrein, and endothelial NOS, has only limited consequences for uterine artery remodeling, fetal growth, and litter size (18, 22, 23). Yet, in middle-aged mice, in which flow-induced vasodilatation reaches an amplitude similar to that in young mice, uterine artery remodeling and fetal survival are simultaneously reduced (19). These observations add to our conclusion that flow-induced vasodilatation and outward remodeling are not directly related (Table 1). The relations between uterine arterial remodeling, uterine blood flow, and fetal growth may help explain the growing body of evidence for a link between intrauterine fetal growth retardation and the risk of developing hyper-

**Fig. 2.** Differential gene expression of the 82-kDa subunit of soluble guanylate cyclase (sGC), thrombospondin-1, tenascin C (TNC), and a desintegrin and metalloproteinase with thrombospondin motifs (ADAMTS1) in rat mesenteric arteries exposed to twice the normal blood flow in vivo. Increase in arterial luminal diameter is statistically significant from that 8 days after exposure to increased blood flow. [Data from Wesselman et al. (57).]

**Fig. 3.** Proposed mechanism whereby a chronic increase in wall shear stress stimulates expression of thrombospondin (TSP-1) and TNC and expression and translocation of the transcription factor Egr-1 as well as activity of matrix metalloproteinases (MMP) and extracellular signal receptor kinases (ERK) and outward remodeling in resistance arteries. FAS, focal adhesion site; CRT, calreticulin.
REFERENCES


