Genetic determinants of vascular remodelling

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Vascular remodelling is an important physiological mechanism that occurs as a result of changes in hemodynamics, and is a pathological process that plays a major role in the clinical manifestations of cardiovascular diseases. Using a mouse model, it was recently established that vascular remodelling is partially based on ligation of the carotid. In this model, low flow was associated with intima media thickening (IMT). IMT is a major manifestation of atherosclerosis of the carotid artery, and it is an important predictor of cardiovascular events. Carotid IMT has a strong genetic component. It was hypothesized that there would be genetically determined differences in outward remodelling and IMT induced by carotid flow alterations. Vascular remodelling among five inbred strains of mice were compared. Despite similar changes in flow in the left carotid among the strains, dramatic differences in remodelling of the partially ligated left carotid relative to control were observed. IMT correlated significantly with heart rate, outward remodelling and changes in plasminogen activator expression, cell proliferation and apoptosis. There were significant strain-dependent differences in the remodelling index (measured as the ratio of vessel area to IMT), which suggest fundamental alterations in sensing or transducing hemodynamic signals among strains. This model should be useful to identify and characterize the role of genes that mediate vascular remodelling.

Key Words: *Carotid artery; Flow; Glagov effect; Mouse; Remodelling*

Les déterminants génétiques du remodelage vasculaire

Le remodelage vasculaire est un mécanisme physiologique important qui se produit par suite de modification de l'hémodynamique. C'est un processus pathologique qui joue un rôle considérable dans les manifestations cliniques des maladies cardiovasculaires. Au moyen d'un modèle de souris, on a récemment établi que le remodelage vasculaire dépend partiellement de la ligature de la carotide. Dans ce modèle, un faible débit s'associait à un épaississement de l'intima et de la média (ÉIM). L'ÉIM est une importante manifestation de l'athérosclérose de l'artère carotide, et c'est un prédicteur important d'événements cardiovasculaires. L'ÉIM de la carotide comporte un élément génétique marqué. On a postulé qu'il y aurait des différences génétiquement déterminées du remodelage vers l'extérieur et de l'ÉIM, induites par des altérations du débit carotidien. On a comparé le remodelage vasculaire de cinq souches pures de souris. Malgré des modifications similaires du débit de la carotide gauche entre les souches, des différences considérables du remodelage de la carotide gauche partiellement ligaturée ont été observées par rapport à celui des sujets témoins. L'ÉIM était corrélé de manière significative avec le rythme cardiaque, le remodelage vers l'extérieur et les changements d'expression des activateurs plasminogènes, de la prolifération des cellules et de l'apoptose. On remarquait des différences marquées propres aux souches dans l'indice de remodelage (mesurées par le ratio entre la zone du vaisseau et l'ÉIM), ce qui laisse supposer des altérations fondamentales de la détection ou de la transduction des signaux hémodynamiques entre les souches. Ce modèle devrait être utile pour repérer et caractériser le rôle des gènes qui soumettent le remodelage vasculaire à la médiation.

Vascular remodelling is an important physiological mechanism that occurs as a result of changes in hemodynamics, and is a pathological process that plays a major role in the clinical manifestations of cardiovascular diseases. In particular, atherosclerosis and hypertension cause remodelling to occur in conduit and resistance vessels. Discussed here is vascular remodelling with a focus on the process of intima media thickening (IMT), an important manifestation of atherosclerosis in the carotid artery. Finally, the important contribution of genetic factors to vascular remodelling, and IMT in particular, has been confirmed as a result of recent human and animal studies (1-4).

VASCULAR REMODELLING DEPENDS ON MANY FACTORS

Several experimental models have been used to investigate the mechanisms by which vessels sense and respond to the stimuli that cause remodelling. Four important general concepts help to explain the findings from these models. First, the vascular bed itself plays an important role in the remodelling response. The formation of a neointima is common in conduit vessels such as coronary, carotid and femoral vessels, but unusual in smaller resistance arteries and the microcirculation. Second, there are large differences among species. Flow-induced neointima is rare in rats, rabbits and pigs, but occurs in mice and humans. Third, the stimulus for remodelling is critical to the response to flow (high versus low versus complete occlusion), injury (endothelial denuding versus severe injury, such as balloon inflation) and coexisting disease (atherosclerosis, hypertension, hyperlipidemia, etc). Finally, genetic contributions are very important in understanding individual responses. For example, different strains of mice and rats have profoundly different responses to changes in flow, and to the effects of atherosclerosis and hypertension. Recent experiments with transgenic mice have identified more than 24 proteins that influence vascular remodelling.

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These experiments indicate that there are many sensors and mediators for vascular remodelling that likely differ in their specific roles based on the four general concepts already discussed.

Three biological laws define vascular remodelling

To understand the mechanisms responsible for vascular remodelling in atherosclerosis, and particularly the biological 'rationale' for IMT formation, we discuss the terminology and the rules that govern remodelling. We believe that the terminology to describe vascular remodelling should be based on simple geometry, ie, terms such as radius, circumference and area. By this, we mean that geometrical changes in the physical compartments of the blood vessel should be used to describe remodelling as exemplified by measurements such as lumen radius (r_{lumen}) and area, and external elastic lamina radius (r_{ext}) and area. We define r_{ext} as equivalent to vessel size. Because changes in wall thickness are important for LaPlace's law, we define wall thickness as $r_{ext} - r_{lumen}$. This terminology is similar to that proposed by Mulvany et al (5) for vascular remodelling in hypertension, although the terminology remains an area of active discussion (6,7).

Normal vascular remodelling is defined by three biological laws that explain the 'rules' that govern the behaviour of organs that exist as fluid-filled tubes. Wolinsky's law states that vessels remodel to maintain constant wall tension. Specifically, Wolinsky and Glagov (8) showed that as the diameter of the aorta increased in mammals (from 0.1 cm in mice to 2.4 cm in pigs), the wall thickness increased proportionally (0.05 mm increase in thickness per 1.0 mm increase in diameter). This compensation in thickness maintained wall tension at a similar value in all species (approximately 2000 dynes/cm^2 for each elastic lamina in the media). Folkow's law states that the reduction in flow that occurs with the vessel contraction is a function of wall thickness (9). Also termed the amplifier effect, this law means that a thicker wall causes a greater loss of lumen than a thinner vascular wall for the same contractile force. Glagov's law states that for the blood flow to remain constant, the lumen diameter must also remain constant. Therefore, vessel size must increase (a greater r_{ext}) if the vessel wall thickness increases. For example, when atherosclerotic plaque develops and the vessel size increases sufficiently, the lumen diameter does not decrease (10). These three laws provide a framework to discuss the molecular mechanisms responsible for remodelling.

Vascular remodelling plays an important role in atherosclerosis and restenosis after coronary interventions in patients

Treatment of obstructive coronary artery disease has focused largely on reducing intimal mass to maintain adequate lumen size (11). However, it is now clear that intimal mass is not the only critical determining factor in stenosis of human coronary arteries (10), because the vessels enlarge to accommodate increasing plaque burden during atherosclerosis and after coronary interventions (12,13). These observations suggest that vascular remodelling preserves blood flow and protects against clinical symptoms associated with stenosis. Data also support a critical role for vascular remodelling after percutaneous transluminal coronary angioplasty, as shown by Cote et al (14) and Tardif et al (15) in the MultiVitamins and Probucol (MVP) study, in which the major effect of probucol was to promote outward remodelling as defined by an increase in r_{ext}.

An important stimulus for vascular remodelling at the site of atherosclerosis is likely to be blood flow itself (16). Because blood flows along a vessel, it creates a force (ie, shear stress) on the vessel wall. During atherosclerosis, there is a loss of lumen diameter that leads to an increase in shear stress. Endothelial cells are uniquely positioned in the vessel to sense changes in shear stress at the plaque site. Compensation by endothelial cells to restore normal shear stress is achieved by their ability to secrete vasoactive factors such as nitric oxide (NO) (17) or growth factors such as platelet-derived growth factor (18-20). Support for a critical role of endothelial cells includes the report by Tronc et al (21) that blocking NO formation with L-nitroarginine inhibited flow-dependent remodelling in rabbits by approximately 70% and data for diminished remodelling in the endothelial NO synthase (eNOS) knockout mouse (18). A recent paper by Stone et al (3) showed that in the presence of high shear stress (τ greater than 38 dynes/cm^2), atherosclerotic arteries remodelled by decreasing plaque area and increasing lumen without changes in vessel size measured by r_{ext}. In arterial sites with low shear stress (τ less than 9 dynes/cm^2), lumen was maintained, despite an increase in plaque size, by an increase in vessel size (r_{ext}). At intermediate values of shear stress (τ betweem 9 dynes/cm^2 and 38 dynes/cm^2), both processes occurred. These data suggest that vascular remodelling with preservation of lumen diameter as described by Glagov can occur in regions with both low and high shear stress, although different mechanisms appear responsible.

Carotid IMT: A marker for pathological remodelling?

An important predictive phenotype for human cardiovascular disease is carotid IMT (23,24). Measuring the IMT may be the best method for detecting early atherosclerosis and assessing the subsequent risk of cardiovascular events (stroke, myocardial infarction and peripheral vascular disease) (24-27). The IMT is measured noninvasively by means of B-mode duplex scanning. The value of IMT is defined by the two parallel echogenic lines that correspond to the lumen-intima and the media-adventitia interfaces. These interfaces are well defined by ultrasound in the far arterial wall only, and it is not possible to distinguish between media and intima. There is a strong association between coronary risk factors and increased IMT, including associations with smoking, diabetes, age, sex (men greater than women), total cholesterol, low density lipoprotein, hypertension and peripheral vascular disease. The most significant positive association is in patients with plaques in the carotid bulb ($P<0.0001$). More importantly, these risk factors predict progression of IMT. For example, in the Monitored Atherosclerosis Regression Study (MARS) (28), dietary cholesterol, body mass index and smoking were significant predictors of the annual progression of carotid IMT ($P<0.05$) in patients with coronary artery disease. In addition, the IMT is highly predictive for cardiovascular events. In the Rotterdam Study, the OR for stroke per SD increase in the carotid IMT (0.163 mm) was 1.41 (25). The European Atherosclerosis Study demonstrated that peripheral vascular disease was significantly associated with an increased carotid IMT (24). Lange et al (29) determined the extent of the familial aggregation of carotid IMT in the presence of type II diabetes. After accounting for the correlation due to age, sex and race, the adjusted heritability estimate for carotid IMT was relatively high at 0.32. These data

provide empirical evidence that subclinical cardiovascular disease has a significant genetic component, and that IMT is a highly predictive and genetically determined clinical measurement.

Flow-dependent remodelling in rats is genetically determined

The phenomenon of flow-dependent vascular remodelling, in which blood vessels enlarge with increased flow and 'shrink' with decreased flow ("outward" and "inward" remodelling [1], respectively), has been observed in vascular development in lambs (30), during enlargement of human maternal vessels in pregnancy (31) and in shrinkage of vessels supplying unused muscle in rats (32). Several laboratories (33-35), including our own, have demonstrated that inward remodelling can be rapidly induced: by two weeks in rabbits (36,37), by four weeks in rats (38) and by two weeks in mice (1,22,39). In rabbits, the diameter reductions were fixed by two weeks and were not reversible with papaverine (37), but were quickly reversed by restoring flow (40). Flow-induced vascular remodelling is endothelial- and age-dependent in rabbits (37,40); vessels that are denuded of endothelium fail to remodel (36). A key role for NO is suggested by the findings that the eNOS inhibitor L-nitroarginine prevents outward remodelling in rabbits' common carotid arteries when stimulated to enlarge by an arteriovenous fistula (21), and mice deficient in eNOS fail to remodel in response to decreased flow (22). As discussed below, transgenic mouse studies have implicated 24 proteins as important for remodelling after complete occlusion of the carotid artery. However, little is known regarding the mechanisms responsible for remodelling in response to increased and decreased flow.

Our laboratory initiated a project to develop a model that would permit an unbiased genetic analysis of the regulatory genes involved in flow-dependent remodelling. Key requirements for our model included the ability to study both high and low flow, reproducibility, technical ease in establishing the change in flow, availability of multiple inbred strains, ability to perform physiological measurements, a sequenced genome with multiple polymorphic markers and low cost. Initially, we focused on rats because their larger size permitted development of the procedure and validation of the remodelling mechanism (41,42).

In Fischer rats, we developed a quantitative, highly reproducible model of carotid flow alteration that involved ligation of the left internal and external carotid arteries, leaving flow only through the occipital artery (42). Left common carotid artery (LCA) blood flow immediately decreased by approximately 93%, whereas flow in the contralateral right carotid artery (RCA) increased by approximately 46%. Changes in shear stress acutely mirrored the changes in blood flow. Outer diameter (OD) increased in the RCA by 28.5%±4.6% in juvenile rats (younger than 12 weeks) compared with 10.8%±4.8% in adult rats (older than 20 weeks), and shear stress returned to initial values after chronic exposure to increased flow in juveniles, but not in adults. There was no difference between juveniles and adults in the response to decreased flow; the OD of the LCA decreased by 15.5%±3.5% in juveniles and by 16.4%±3.4% in adults. We found that there was a direct monotonic relationship between flow and diameter; decreases in flow reduced OD in all rats, and increases in flow enlarged OD. A difference in the sensitivity to flow (of borderline significance) was observed between juvenile and adult carotids that were exposed to an increased flow, with greater remodelling occurring in juveniles than in adults for a given change in flow. Medial cross-sectional area increased in the RCA of juvenile rats with increased flow, but there was no change in media area in the LCA. No intima formed in either high- or low-flow vessels. We found that eNOS levels in ligated rats increased significantly in the RCA and decreased in the LCA compared with sham rats.

Next, we compared the remodelling changes in four strains of inbred rats (41). After four weeks of altered flow, there were significant interstrain differences with respect to the change in the OD of the carotid, the relationship between flow and shear stress, and the extent to which shear stress was normalized. Genetically hypertensive rats exhibited the greatest reduction in shear stress in response to increased flow, stroke-prone spontaneously hypertensive rats exhibited a smaller response and brown Norway rats exhibited the smallest response. Stroke-prone, spontaneously hypertensive rats and genetically hypertensive rats also differed significantly in outward remodelling in increased flow arteries. In response to decreased flow, brown Norway rats exhibited the smallest reduction in shear stress. These findings demonstrate significant strain-dependent differences in shear stress regulation and vascular remodelling in response to altered flow. Like our previous study (42) in Fischer rats, there was no change in media area and no neointima formation. However, we have chosen mice for our genetic analysis because the magnitude of the phenotypic differences (measured by changes in vessel wall components) was much greater.

Flow-dependent remodelling in mice is genetically determined

Using a complete ligation and flow cessation mouse model (43), the roles of at least 24 genes in geometrical remodelling have been studied in genetically altered mice (eNOS, endothelin receptor, Fas ligand, matrix metalloproteinase [MMP]-2, MMP-9, neuronal NOS, neurite outgrowth inhibitor B, p130, p22phox, p75 netrophin receptor, plasminogen activator [PA] inhibitor-1, steroid receptor coactivator-3, tissue factor pathway inhibitor, thioredoxin reductase-3, vimentin, vitronectin, adenosine 2a receptor, inducible NOS, osteopontin, P-selectin, toll-like receptor-4 and von Willebrand factor) (44-64). Vascular remodelling is genetically controlled in the complete occlusion model as measured in inbred mice strains (2). Specifically, significant differences in variations in vessel size, neointima formation, lumen area and medial thickness were found. The largest changes occurred in SJL/J mice, which displayed extensive inward remodelling leading to a 78% decrease in lumen area. Lumen narrowing was also impressive in FVB/NJ mice and was largely due to extensive neointima formation as a result of vascular smooth muscle cell proliferation. These studies suggest that the mouse carotid provides an ideal model to study the genetics of IMT and fundamental mechanisms of vascular remodelling.

We used the same partial ligation model developed for the rat to study flow-dependent remodelling in the mouse (1,39,65). More importantly, the presence of IMT and lumen narrowing in the mouse suggested that the mouse carotid might be useful to study the 'Glagov phenomenon'. This refers to the observation that in the early stages of atherosclerosis, coronary arteries enlarge in relation to plaque area to preserve lumen, thereby obeying Glagov's law (10). Recent clinical data demonstrate that regions of low shear stress develop

progressive atherosclerosis measured by increased IMT and outward remodelling of coronary arteries (3). Thus, we anticipated that the low-flow LCA may develop neointima that would be modulated by genetic factors.

We compared vascular remodelling among five inbred strains of mice (C3H/HeJ [C3H], DBA/2J [DBA], C57Bl/6J [C57], FVB/NJ [FVB] and SJL/J [SJL]) (39). Despite similar changes in flow in the LCA among all the strains, we observed dramatic differences in remodelling of the partially ligated LCA relative to the control. The smallest IMT was found in C3H/HeJ mice, moderate changes were noted for C57 and DBA mice, while the largest IMT volumes were in SJL/J and FVB/NJ mice (3.5-fold greater than C3H mice). Shear stress did not differ among strains after ligation. Among hemodynamic factors, low shear stress and high heart rate (HR) were predictive for IMT. Specifically, HR (C3H=592±6 beats/min, SJL=649±6 beats/min and FVB=683±7 beats/min), but not systolic blood pressure (C3H=116±2 mmHg, SJL=119±1 mmHg and FVB=136±1 mmHg), was predictive. The most impressive finding was a strong correlation between IMT and outward remodelling (r_{ext}) among inbred strains. Of interest is that, despite a significant correlation of external elastic lamina with IMT, there were also significant strain-dependent differences in remodelling index (measured as a correlation slope). Specifically, among the high remodellers, SJL mice remodel mostly by increased intima formation, while FVB mice remodel primarily by increased media. We also observed significant differences between these two strains in the remodelling index consistent with a limitation of outward remodelling in SJL mice compared with FVB mice. The remodelling index provides insight into genetic differences in vascular remodelling, which suggests fundamental alterations in sensing or transducing hemodynamic signals among strains. Because shear stress changes did not differ among strains, a primary role for cells in the vessel wall rather than endothelium is suggested for the early stages of vessel remodelling.

The RCA underwent outward remodelling in response to increased shear stress (from 80×10^{-6} μm^3 to 100×10^{-6} μm^3) with increased lumen volume (from 60×10^{-6} μm^3 to 80×10^{-6} μm^3) as expected (66). High shear stress was atheroprotective for the RCA in that there was no IMT similar to human coronary arteries (3). Genetic factors appeared to play a small role in the response to high shear stress because there were no significant differences among inbred mice strains.

To gain insight into some of the events that may be occurring in the vessel wall, we first studied the role of the two major matrix-degrading systems, PAs and MMPs. Specifically, we compared the expression of urokinase-PA, tissue-PA, MMP-2 and MMP-9 in ligated carotids of C57 and FVB mice (65). Among PAs and MMPs, increased expression of tissue-PA and urokinase-PA correlated very significantly with increased IMT. MMP-2, MMP-9 and tissue inhibitors of MMP-2 expression also increased, but did not differ between strains. Based on these findings, flow-induced IMT of the mouse carotid correlates with tissue-PA and urokinase-PA expression in two inbred mouse strains.

RELEVANCE TO HUMAN DISEASE

We believe that the partial carotid ligation model of vascular remodelling in the mouse exhibits features similar to human atherosclerotic arteries characterized by Glagov et al (10). At the early stages of atherosclerosis, coronary arteries enlarge in relation to plaque area to preserve lumen until plaque area occupies approximately 40% of the vessel area (10). Of interest, the common carotid, coronary and renal arteries are more likely to enlarge in response to plaque formation than the iliac and femoral arteries (67). In our recent study (1), there was a strong correlation between outward remodelling and IMT formation in the carotid. A similar correlation was previously reported for vessel and intima areas in the carotid flow cessation model in hypercholesterolemic *ApoE* knockout mice (68). Also of importance, we found that genetic background is a critical determinant of remodelling with unique responses among inbred strains.

Hemodynamic factors other than shear stress, such as HR and blood pressure, may also be important for vascular remodelling. The HR was previously shown to correlate with IMT in experimental (69,70) and clinical (71) studies. A strong relationship between HR-corrected QT interval and IMT was found in humans with early atherosclerotic disease (71). Despite HR differences between mice and humans, it was shown in humans in vivo that HR increases in physiological ranges were associated with a reduction in arterial compliance (72). Finally, HR reduction by sinoatrial node ablation in cynomolgus monkeys decreased plaque burden in coronary (69) and carotid arteries (70).

SUMMARY

We have developed an animal model of human vascular disease that has a strong genetic basis. Among hemodynamic factors, low shear stress and high HR were predictive for IMT in inbred mice strains. In addition, our findings suggest that fundamental changes in the ability of vessel wall cells to sense or transduce hemodynamic signals are important genetic determinants of remodelling. Approximately 40% of the variability in the carotid IMT was shown recently to be dependent on family history (73). To date, association studies of polymorphisms and IMT have not yielded strong candidate genes (4,74). We propose that future experiments using quantitative trait locus analysis of a cross involving strains in the present study (ligated C3H × SJL or C3H × FVB strains) will be useful to elucidate mechanisms of outward remodelling and IMT in response to flow reduction.

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