Dynamics of Shear-Induced Redistribution of F-Actin in Endothelial Cells In Vivo

B.L. Langille, J.J.K. Graham, D. Kim, and A.I. Gotlieb

The steady-state responses of endothelial cell F-actin distribution to changes in in vivo shear stress have been well documented. The purpose of the current work was to define the dynamics of redistribution of F-actin in the period immediately after experimental changes in shear. We used abdominal aortic coarctation in rabbits to experimentally increase shear stress downstream from the coarctation by approximately twofold. In situ staining was employed to track subsequent F-actin redistribution. Within 12–15 hours, the number of stress fibers in the central regions of the cells decreased, and some separation of junctional actin in adjacent cells occurred. Long, central stress fibers of variable thickness were evident at 24 hours, but the band of actin normally seen at the periphery of the cells could no longer be distinguished. The redistribution of F-actin was completed over the next 24 hours by an increase in thickness of central stress fibers. Restoration of normal F-actin distribution after coarctations were removed proceeded more slowly. The long, thick stress fibers that were induced by high shear were replaced by thinner or shorter microfilament bundles 48 hours after the coarctations were removed. At 72 hours, central stress fibers were primarily long, thin structures. Peripheral F-actin was not fully restored at this time. Peripheral F-actin was restored 1 week after removal of the coarctation, but there were still more and longer stress fibers at this time than were observed in control aortas. If current hypotheses linking central stress fibers to cell–substrate adhesion and peripheral actin to permeability regulation are correct, then our data indicate that induction of high hemodynamic shear stress may transiently compromise substrate adhesion. Subsequent alterations may enhance substrate adhesion, although intercellular permeability may be altered due to reductions in peripheral actin. (Arteriosclerosis and Thrombosis 1991;11:1814–1820)

The localization of fibrofatty plaques at arterial bends and branch points suggests a role for hemodynamics in the pathogenesis of atherosclerosis.1–3 In vivo studies of hemodynamic shear stress have shown that the shape and orientation of endothelial cells is determined primarily by blood flow.4–7 In vitro studies have demonstrated that flow-related shear stress alters several aspects of endothelial cell structure and function, including cytoskeletal organization,8 histamine9 and tissue plasminogen activator synthesis,10 endocytosis,11 and the status of K+ channels.12 Some alterations in vasomotor tone are mediated by the effects of shear stress on the endothelium through the release of agents such as endothelium-derived relaxing factor,13 prostaglandins,14 and possibly endothelin.15,16 In addition, shear modulates arterial structure through endothelium-dependent remodeling of the vessel wall.17–19

The F-actin microfilament system, a component of the cytoskeleton, is important to the endothelial cell because of purported roles in the control of cell adhesion, cell migration, maintenance of cell shape, and cell permeability.20 In situ, F-actin is generally present as a continuous band around the periphery of cells and in microfilament bundles, or “stress fibers,” in the central portion of cells.21–23 Some studies have reported that the central microfilament bundles in endothelial cells are more numerous in regions of the arterial vasculature exposed to elevated shear stress.21–24 Kim et al25 reported profound alterations in F-actin microfilament organization in endothelial cells at sites of elevated shear levels in the normal rabbit aorta. The peripheral microfilament band was disrupted, whereas the central stress fibers were markedly increased in thickness and length. The hypothesis that alterations in hemodynamic
shear stress in vivo cause this reorganization of the F-actin microfilament system was supported by studies in which a 60% coarctation was used to alter shear stress in the midabdominal aorta of the rabbit. This procedure produces a region of moderately elevated shear downstream from the stenosis, in which peripheral F-actin was dispersed and large central stress fibers were formed. These findings indicate that in vivo actin microfilament distribution can be modulated by experimentally altering flow conditions. These findings are consistent with evidence of the influence of shear on F-actin distribution in cell culture systems.

To date, all in vivo studies have examined the steady-state responses of F-actin distribution to shear stress, that is, 2–3 weeks after shear is altered. While the steps that lead to these steady states are unknown. The purpose of our study was to characterize the dynamics of the F-actin response to experimental alterations in hemodynamic shear stress in vivo. The nature and speed of cytoskeletal changes were studied with the coarctation model. We also examined the restoration of a normal F-actin distribution after coarctations were removed and after normal shear stresses were restored.

Methods

Experiments were performed on 28 male New Zealand White rabbits weighing 2.5–3.5 kg. All animals were fed a standard rabbit chow diet (Ralston Purina, Woodstock, Canada).

Coarctation

Animals were prepared for surgery as previously described. Briefly, anesthesia was induced with an intramuscular injection of 1 mg/kg body wt xylazine and 45 mg/kg body wt ketamine. The abdominal aortas were exposed via a midline incision and were constricted with Ethicon 6-0 silk midway between the left renal artery and the iliac bifurcation. During this procedure, femoral arterial pressure was measured with a Statham P23 ID transducer connected to a Gould chart recorder (Gould Inc., Glen Burnie, Md.). The ligature was tightened slowly until a decrease in diastolic blood pressure was first observed, at which point pulse pressure was almost obliterated. Subsequent vascular casts indicated that this procedure consistently reduced luminal diameter by about 60%. In sham surgeries, a nonconstricting suture was tied loosely around the aorta.

Cytoskeletal Changes After Coarctation

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Perfusion fixation has been described elsewhere. Briefly, animals were given 1,000 units heparin (Organon Canada Inc., Toronto, Canada), and then killed with the euthanizing agent, T-61: 200 mg/ml N-[2-(m-methoxyphenyl)-2-ethylbutyl-(1)]-hydroxybutyramide, 50 mg/ml 4,4′-methylene-bis-cyclohexyltrimethylammo-
Sequential Changes After Coarctation

The most pronounced changes at each time point occurred approximately 8 mm distal to the coarctation. The first of these were observed after 12–15 hours. Cells contained fewer stress fibers than in the aortic endothelium of either sham-operated or control animals, and many cells were nearly devoid of central F-actin (Figure 2, left panel). However, some regions contained very long, thin stress fibers that were generally oriented parallel to the long axis of the cell (Figure 2, right panel). The undulating stress fibers described above (Figure 1, right panel) were not observed at this time. In addition, two bands of F-actin were observed at the junctional regions of some cells, suggesting separation of the stained regions of adjacent cells (Figure 2, right panel). Often, one of the adjacent bands was more like a dense peripheral bundle, whereas the other was a thinner structure. Occasionally, peripheral staining was also less intense in focal areas throughout the zone. We frequently observed cells in which several of these stress fibers projected from a single site at the upstream and sometimes the downstream extremity of the cell (Figure 3).

By 24 hours, cell boundaries were no longer identifiable by peripheral actin staining. Central stress fibers were much longer than in control aortas, although their thickness was highly variable (Figure 4). The thicker stress fibers often exhibited the waviness described above. As described previously, we observed stress fibers in these high shear regions that appeared to extend from the nucleus in one cell to the nucleus of a neighbor (Figure 4). This finding suggests that stress fibers in adjacent cells are occasionally in register at the cell junction. There were some differences between the patterns seen at 24 and 48 hours in that there were more long, thicker stress fibers at the later time. F-actin distribution at 48 hours was not distinguishable from that seen at later times (see Figure 1, right panel).

At 4 mm downstream from the stenosis, F-actin redistribution proceeded more slowly and was ultimately less pronounced than at 8 mm. Slower, more modest responses nearer to the coarctation suggest that the changes are not strongly influenced by artifacts associated with surgically induced coarctation. At 15 hours, there was a general reduction in central F-actin, although a small number of thick,
medium-length stress fibers were observed. These became more numerous by 24 hours, but peripheral staining never disappeared (Figure 5). We did not observe instances where several stress fibers appeared to emanate from the upstream limit of cells, as was seen at 8 mm.

**Dynamics of the Response to Restoration of Normal Flows**

Vascular casts demonstrated that normal aortic geometry was restored by 1 hour after removal of the coarctation. Forty-eight hours after the coarctation was removed, many of the long, thick stress fibers characteristic of zone 3 were replaced by long and thin or short and thick stress fibers (Figure 6, upper left panel). By 72 hours, very few of the very thick stress fibers remained; instead, long, thin fibrils became predominant (Figure 6, right panel). Peripheral staining at the cell boundaries was not detectable at this time. Peripheral staining was only fully reestablished at 1 week (Figure 6, lower left panel). There were many thin stress fibers that were generally longer than those seen in control vessels; thus, normal F-actin distribution had not returned fully at this time. The endothelial cell shape resembled that seen in control animals (Figure 6, lower left panel).

**Discussion**

The most striking adaptations of the endothelial F-actin microfilament system to elevated shear stress
are the formation of very large (giant) stress fibers that extend much of the length of the endothelial cells and that frequently appear to be in register across cell boundaries and the loss of F-actin from the cell boundaries. The current study has revealed that these adaptations proceed through an orderly series of interrelated events. First, there was a loss of many of the short, central stress fibers observed in most arterial endothelium. A coincident decrease in staining at the cell periphery was characterized by the frequent appearance of two distinct bands of F-actin. Often, one band was prominent and the other, thinner. Presumably, one band was associated with each of the two adjacent cells. It was not clear whether this represented physical separation of actin from the cell–cell junction or whether a decrease in staining intensity allowed resolution of two bands that remained in a fixed position. In any event, it appears that the pool of F-actin in the cell is diminished early in the response. Our studies do not indicate whether this diminution reflects F-actin degradation or redistribution to the cellular pool of G-actin. It is tempting to suggest, however, that this transient phase of decreased F-actin represents a window of vulnerability of the cell to mechanical trauma, given the putative role of F-actin in cell–cell and cell–substrate adhesion. Physiological or pathophysiological changes in the delivery of blood flow to arterial branches will cause changes in shear stress near branch orifices that are probably comparable to those we induced, and a consequent dispersal of F-actin may render endothelium at these sites susceptible to injury.

This initial phase was followed by the formation of very large cytoplasmic stress fibers. It was striking that this step was preceded, as noted above, by the disappearance of stress fibers from many cells. We infer from this observation that many or all of the giant stress fibers that develop in the second phase arise de novo from nucleation sites, rather than as a result of extension of preexisting microfilament bundles.

When stress fibers reappeared, either short and thick or long and thin bundles were observed. The first of these early forms suggests that the giant stress fibers can grow through synchronous polymerization of many adjacent microfilaments. The second form suggests an initial lengthy extension of microfilaments, which is then followed by addition of microfilaments parallel to these. Such remodeling of stress fibers is likely carried out by actin-binding proteins that are able to affect the three-dimensional architecture of microfilament bundles and to control the linear assembly of actin.

Stress fibers are thought to ensure integrity of the endothelial lining when it is exposed to high shear forces, and some evidence from tissue-culture studies supports this hypothesis. Wechezak et al. showed that substrate adhesion of endothelial cells in a shear field is impaired if the cells are treated with cytochalasin B. Observations by Sato and coworkers provide a conceptual framework for this finding. They found that endothelial cells conditioned by shear
stress exhibit decreased deformability, whereas microfilament-disrupting agents increase deformability. Presumably, decreased cell deformability may enhance resistance to shear damage, perhaps by distributing stresses more evenly throughout the cell. Satcher et al.\(^3\) raise another interesting possibility. They suggest that if stress fibers are associated with focal contacts with substrate at their upstream ends, as Wechezak et al.\(^3\) have observed in vitro, then they can serve to "moor" the cells that are exposed to shear forces. Shear forces imposed on the apical surfaces of the cells would then translate into tension in stress fibers that is transmitted to substrate adhesion sites. It is also of interest that, early after coarctation, several stress fibers frequently appeared to emanate from a single site located at the upstream limit of the cell. If these stress fibers originate from a common point on the basal surface, then this could be a major site of substrate adhesion. Sometimes, however, these structures were also observed at the downstream ends of the cells. Previously, White et al.\(^3\) demonstrated a preponderance of stress fibers at the upstream ends of cells that showed some similarity to these structures, although a common origin was not apparent. It may be that they observed a later manifestation of the transient pattern that we found.

Our morphological data cannot add further to how these hypotheses concerning substrate adhesion apply to the short stress fibers seen under relatively low shears; however, it does suggest that additional mechanisms are involved at high shears. Our in situ observation that many stress fibers in adjacent cells are in register, creating the illusion that single microfilament bundles extend from one cell to another, suggests that stress fiber functions are integrated at the intercellular level. The most likely scenario is that these stress fibers emanate from adjacent nucleation sites at junctional complexes and project upstream in one cell and downstream in the neighboring cell. If so, it is unlikely that they associate with the basal surface in both cells to moor the endothelium to substrate in the manner described by Satcher et al.\(^3\). However, if one function of stress fibers is to distribute stresses throughout the cell, then this concept may extend to intercellular stress distribution via these larger structures. Thus, they may limit deformation of the endothelial monolayer over large areas, or it is possible that they tether cell junctions that are exposed to high deforming stresses.

Reversion to a normal F-actin distribution after removal of the coarctation proceeded more slowly than the initial response to coarctation, with responses requiring 7 days rather than 48 hours to go to completion. Apart from the time course, the process was largely the reverse of that seen after coarctation. However, we did not see instances of several stress fibers emanating from a single site, as occurred transiently after coarctation. If, during assembly, several stress fibers first emanate from a single nucleation site when shear is elevated, then there may be neither a mechanism nor an adaptive advantage for their reassociation with such sites during depolymerization.

Taken together, these findings indicate that local increases in shear stress induce an orderly, reversible reorganization of cellular actin that is most pronounced and most rapid when shear is most elevated. The process appears to involve an early decrease in the pool of F-actin in the cell, followed by the preferential repolymerization of F-actin.

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