Do endothelial injuries predispose to atherosclerosis?

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Summary

Cynomolgus monkeys were subjected to mid-thoracic aortic coarctation by suture plication in order to produce local medial and endothelial injury and a local increase in flow velocity and shearing stress. One group was fed an atherogenic diet containing 2% cholesterol and 25% peanut oil for a period of six months, while the remaining animals were fed monkey chow. Aortas were fixed by controlled pressure perfusion in situ and opened by means of two parallel longitudinal cuts in order to minimize distortions due to bending of flattening. Luminal surfaces were examined by gross inspection and by scanning and transmission electron microscopy. Zones of high shear stress, such as the narrowed segment of the coarctation channel and the rims of flow dividers about ostia were free of intimal plaques in hypercholesterolemic animals despite extensive proximal and distal involvement. Lesions developed proximal and distal to ostial flow dividers including ostia within the otherwise spared coarct channel. There was no evidence of endothelial injury or disruption within the aortic constriction or at the rims of flow dividers. Suture disruptions of the endothelial surface within the coarctation channel were completely healed and spared of atherosclerosis. These and other recent findings concerning endothelial integrity over early lesions, and the requirement for intact endothelium before formation of plaques, indicate that lesions tend to form where shearing stress is relatively low and that endothelial disruption appears to complicate rather precede plaque formation. Elevated shearing stress is not necessarily injurious to endothelium and may inhibit atherogenesis.

Zusammenfassung

Sind Endothelschäden prädisponierend für Atherosklerose?

ten würden. Nahtläsionen an der Endotheloberfläche innerhalb des Einengungskanals der Aorta waren voll ausgeheilt und von Atherosklerose ausgespart. Diese und andere kürzlich mitgeteilten Ergebnisse, die die endotheliale Integrität bei sog. frühen Läsionen betreffen sowie die Notwendigkeit für ein intaktes Endothel bevor sich atherosklerotische Plaques bilden, zeigen, daß sich Läsionen vornehmlich dort bilden, wo der Schärfstreß relativ gering ist und daß endotheliale Zerstörung eher als Komplikation erscheint, als daß sie Vorläufer der Plaquebildung ist. Erhöhter Schärfstreß muß nicht notwendigerweise zum Endothelschaden führen und könnte die Atherogenese sogar inhibieren.

Introduction
Increased shearing stress due to local increases in blood flow velocity or turbulence, has been considered to be injurious to endothelium and experimental devices designed to increase shearing stress have produced endothelial disruptions (1) and increased endothelial permeability (2). Such changes are considered to predispose to increased intimal ingress of blood borne lipids and mitogens, and to the subsequent formation of atherosclerotic plaques (3). Aortic ostial flow dividers are regions of relatively high shear stress and a number of investigators have reported distortion, disruption and detachment of endothelial cells at, and immediately distal to, these structures 4, 5, 6, 7, 8. They have suggested that these changes are evidences of endothelial injury by elevated shear stresses and that these sites are vulnerable to plaque formation. We sought to produce abnormal increases in shear stress by means of a local constriction of the thoracic aorta in cynomolgus monkeys. Since the procedure included plication and suturing of the aorta, we also produced a transmural and endothelial injury at the site of coarctation. We evaluated the coarctation channels, suture sites and aortic ostia for evidences of endothelial injury and determined the pattern of localization of diet induced atheromatous lesions in perfusion fixed aortas. We saw no evidences for a correlation between increased shear and endothelial injury, and no evidence for a relationship among increased shear, endothelial injury and plaque localization. On the contrary, zones of increased shear were spared of atherosclerosis.

Methods
Twelve male cynomolgus monkeys were studied. Six animals underwent coarctation of the mid-thoracic aorta by suture plication. Blood pressure was monitored in the brachial and femoral arteries and an effort was made to achieve a 50% narrowing of aortic internal diameter and a mean aortic pressure gradient across the stenosis of 20 to 40 mm Hg at the time of surgery. Three monkeys with coarctation and three without coarctation were fed an atherogenic diet containing 2% cholesterol and 25% peanut oil for a period of six months, while three with coarctation and three without were fed a standard laboratory diet. At the time of sacrifice, each aorta was fixed in situ by perfusion with buffered gluteraldehyde at a pressure of 80 to 100 mm Hg. The aorta was then excised and opened by means of two parallel longitudinal cuts in order to avoid bending, flattening or other distortions of the configuration of the coarctation channel or the ostia. The luminal surface of the aorta was evaluated for evidence of endothelial injury and for atheromatous plaque localization by gross inspection, by light microscopy and by scanning and transmission electron microscopy. Particular attention was paid to the coarct channel, to the aorta immediately above and below the coarctation channel, to the flow dividers of the celiac, superior mesenteric, renal and intercostal artery branches, and to intercostal artery ostia located within the coarctation channel.

Results
Animals without coarctation and on a normal diet were found to have an intact endothelial lining throughout. Endothelial cells were oriented with their long dimensions in the direction of flow. About ostia, endothelial cells were elongated parallel to presumed lines of flow and became rounded without definite orientation in the lateral recesses on the branch side of the flow divider. There was no evidence of desqua-
formation, disruption, fusiform deformation or other endothelial damage at or about the aortic ostial flow dividers (Fig. 1). When occasional endothelial disruptions were seen, these could be related to handling or preparation artifacts and bore no consistent relationship to ostia. Coarctation of the aorta did not result in endothelial changes within the coarct channel or about aortic ostia, either proximal or distal to the coarctation. Sutures were evident, often projecting from the intimal surfaces where the plication had been performed. The overlying endothelium was however intact and smooth. In addition, there was no evidence of intimal thickening in relation to the suture material.

Animals fed the atherogenic diet developed prominent intimal foam cell lesions. The extent and severity of these lesions was variable. Lesions consisting of a single layer of intimal foam cell were always covered by intact endothelium. Evidences of endothelial disruption were noted only over extensive, multilayered, confluent complex foam cell lesions. Several patterns of lesion localization were noted about ostia with a predominance of lesion development at the upstream and lateral rims (Fig. 1). These deposits were sometimes confluent with adjacent aortic plaques and with plaques which extended into the branch vessels, but the rim of the flow divider was not only free of endothelial

**Fig. 1**
(A) Aortic ostial flow divider. Endothelial cells are intact with no disruption (X 2500). (B) Foam cell lesion on proximal rim of intercostal ostium. Rim of flow divider is free of lesion formation (X 1000). Arrow denotes direction of flow.

**Fig. 2**
(A) Abrupt cessation of foam cell lesion at coarctation channel. The endothelial surface is intact throughout (X 1000). Transmission electron microscopy demonstrates normal, intact endothelium within coarctation channel (X 5000). Arrow denotes direction of flow.
damage, but was always free of intimal foam cell lesions despite extensive proximal and distal involvement.

The coarctation channel in diet fed animals was lined by intact endothelium with no evidence of endothelial disruption or distortion and the coarctation channel was almost entirely free of foam cell lesions despite extensive proximal and distal lesion deposition (Fig. 2). The pattern of lesion deposition appeared to correspond to flow velocity patterns. Zones of high flow velocity, including jets distal to the coarctation, were spared, while areas of relative stasis, immediately proximal and distal to the plication, were most involved (Fig. 3). Despite complete sparing of the adjacent aorta within the channel, occasional ostia within the coarct channels were involved by lesions similar in distribution to those seen about ostia elsewhere (Fig. 3).

Discussion

A number of investigators have reported distortion, disruption and detachment of endothelial cells at and immediately distal to, aortic ostial flow dividers in rabbits 4, 5, 6, 7, 8. We found no evidence of endothelial disruption at aortic ostial flow dividers in our carefully handled monkey aortas. We have previously shown that in rabbits the spindle cell deformation of endothelial cells noted by other investigators could be prevented by bivalving the fixed aorta and avoiding maneuvers that would distort the configuration of the branch ostia (9). We could consistently produce endothelial distortion on the rim of aortic ostial flow dividers by opening the aorta with a single longitudinal cut and pinning it flat. Nor did we find any evidence of endothelial injury in the region of high flow velocity and shear within the stenotic channel or at the sites of previous suture injury where the aorta was plicated to produce narrowing. The levels of shear stress which produced endothelial injury in Fry's experiments (1) were probably much higher than were present in our coarctation channels. While it is possible that the high shear rates used by Fry could be attained under some conditions in the intact animal, there is little evidence that this actually occurs under normal circumstances. In other experiments in our laboratory, we showed that the endothelium heals within a few days following injury (10). Since the monkeys which form the basis of this report were sacrificed six months after surgery, it is not surprising that the surface over the suture plication was entirely healed. Neither the previous endothelial or medial injury, nor the deformity associated with the presence of the suture resulted in plaque formation.

The areas of presumed relatively high shear stress at ostia and in the coarct channel were remarkably free of intimal plaques. Indeed, it appeared that plaques tended to develop inste-
ad in the zones of relatively low flow velocity and low shear stress immediately below the aorta, above the coarctation channel, and in the proximal rims of the ostia. Other investigators have implicated low shear stress 11, 12, 13, rather than high shear stress as the more important predisposing hemodynamic factor in atherogenesis. Our findings would tend to support this viewpoint. It should be noted, however, that higher than normal shear rates, such as those which obtained in the coarct channels, actually protected the aorta from plaque formation. These results would seem to suggest that interference with distal flow due to increased peripheral resistance or critical stenosis may favor atherogenesis in the proximal vessel segments where flow is reduced. Conversely, exercise and therapeutic interventions designed to increase distal flow or decrease peripheral resistance could be expected to retard atherogenesis in proximal vessels.

We have previously reported that endothelium is conserved in hypercholesterolemic monkeys away from plaques and over single layered plaques and that disruption of endothelium occurs only over thick plaques (14). The observations reported here lend further support to the notion that endothelial injury and disruption is not a necessary condition for plaque formation and may indeed be a complication of plaque formation. The role of endothelial integrity in atherogenesis requires more detailed study. Precise correlation of hemodynamic variables with plaque induction and evolution could provide new insights into this critical problem.

References

6 BJÖRKERUD, S. and BONDJERS, G.: Endothelial integrity and viability in the aorta of the normal rabbit and rat as evaluated with dye exclusion tests and interference contrast microscopy Atherosclerosis 15: 285–300, 1972