Effects of Thrombosis on Vascular Tone in Rat Mesenteric Arteries With Endothelium In Vivo

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We examined the effects of thrombosis on vascular tone in vivo, using small mesenteric arteries of rats (about 300 μm i.d.) and a video recording system attached to a microscope. To induce thrombosis we damaged the vessel wall over a short segment by compression and exposed the media to the bloodstream. A thrombus mainly consisting of platelets gradually enlarged at the damaged site and caused 50–100% narrowing of the lumen. The diameter at the site downstream from the thrombus did not change as long as the blood flow was not interrupted. Instead, vasoconstrictor response to serotonin (3.0 and 30 μg/ml) or norepinephrine (0.1, 0.3, and 1.0 μg/ml) given topically was significantly inhibited at the site downstream from the thrombus compared with that at the upstream site (p<0.01). Nonthrombotic mechanical narrowing of the vascular lumen did not affect vascular constriction by these agents. After damage to the endothelium by intra-arterial infusion of a detergent (0.3% CHAPS), the inhibition of the constrictor response by the thrombus was no longer observed. Once the thrombus occluded the vascular lumen and the blood flow was interrupted, the diameter at the downstream site declined markedly, from 291±22 to 77±21 μm (mean±SEM, n=7, p<0.01) even without endothelial damage. Mechanical nonthrombotic occlusion of the vascular lumen induced only a small diameter reduction to 241±16 μm. We conclude that a partially occlusive thrombus may release a material that inhibits vascular constriction by serotonin or norepinephrine through an endothelium-dependent mechanism and that an occlusive thrombus induces constriction of the downstream vascular bed even with endothelium. (Circulation Research 1990;67:312–318)

During thrombus formation, aggregating platelets release various vasoconstrictor agents including serotonin and thromboxane A$_2$, which has been thought to constrict the arterial smooth muscles and aggravate tissue ischemia in vivo. Endothelium attenuates constrictor response of vascular smooth muscles to various vasoactive agents or reverses it to relaxation by releasing endothelium-derived relaxing factor(s) both in vitro and in vivo.$^{3-6}$ Aggregating platelets themselves are reported to induce coronary contraction in vitro, which is inhibited by endothelium or is reversed to relaxation when the artery is precontracted.$^7$ These results suggest that the effect of thrombosis on vascular tone in vivo will also be substantially modified by endothelium.

In addition to platelets, thrombosis in vivo involves the blood coagulation system as well as leukocytes, which may also affect vascular tone,$^{8,9}$ and the net effect of thrombosis in vivo on arterial tone may be different from the results obtained in studies in vitro with aggregating platelets. The state of blood flow also influences vascular tone in vivo through an endothelium-dependent mechanism.$^{10,11}$ The purpose of this study was to elucidate the response of arteries with intact endothelium to thrombus formation in vivo. We employed a microscope–video recording system that allowed continuous and thorough observations of both thrombus formation and vascular tone in small mesenteric arteries of rats in vivo.$^{12}$

Materials and Methods

Animal Models

Male Wistar rats weighing 220–280 g were anesthetized with an injection of 60 mg/kg i.p. pentobarbital. The right jugular vein was cannulated with polyethylene tubing for the administration of additional pentobarbital to maintain the level of anesthe-
sia. After a small midabdominal incision, a part of ileum was pulled out and spread over the transparent warm (37°C) stage. Fat and connective tissues surrounding the mesenteric artery (approximately 300 μm i.d.) were carefully removed under a dissecting microscope. In some rats we cannulated a polyethylene tubing into the side branch of the mesenteric artery to use for intra-arterial drug administration (Figure 1). The preparation was mounted on a biological tricocular microscope (Optiphot, Nikon, Tokyo, Japan) attached to a color video recording system (model GP 5J, Hitachi, Tokyo, Japan) or, in some cases, to a camera (model M35S, Nikon). The surface of the mesenteric artery was superfused continuously with warm Tyrode’s solution at a rate of 1 ml/min. Magnification with the microscope was ×40 or ×100.

To induce thrombosis, we damaged a short segment (about 300 μm) of mesenteric arterial wall by compression and exposed the media or, in some cases, the adventitia to the bloodstream (deep vessel injury). We used a heat-blunted glass micropipette (tip diameter of approximately 200 μm) attached to a micromanipulator for the compression vascular damage and applied 20–25 g at the tip of the glass micropipette repeatedly for 20–30 times. This caused passive dilation of the segment, and in some cases, a small amount of bleeding ensued. The damaged segment lost responsiveness to vasoactive agents. Histological examination revealed a loss of endothelial layer, disruption of internal elastic lamina, and a thinning of media almost over the entire circumferential length of the segment (Figure 1).

After vascular damaging by compression, a thrombus, mainly consisting of aggregating platelets, developed at the damaged site and gradually increased in size. This process resulted in 50–100% narrowing of the vascular lumen. Repetitive compression over the entire circumference of the vascular segment usually induced thrombus formation of a larger size, which frequently occluded the vascular lumen and stopped the blood flow. The cessation of flow was easily recognized by observing stagnant blood cells within the vascular lumen. The thrombus broke down spontaneously within a few minutes after attaining each maximum size. The thrombus formation was repeatedly observed more than five times over 30–60 minutes after compression damage. Such thrombus formation was reproduced by additional compression damage at the same vascular segment.

**Measurement of Diameter Changes**

Changes in internal diameter were measured on a playback display of the recorded videotape or on a photograph, using calipers with a videotaped or photographed stage micrometer for reference. Drug response of mesenteric arteries with thrombus formation was analyzed by measuring diameter changes at one point in each upstream and downstream site (approximately 1,000 μm from the thrombus), because the diameter changes were almost homogeneous over the observed segment at each site. Reproducibility of the measurement was examined by correlating the data between repeated measurements and between different observers. The correlation coefficient was 0.993 (p<0.001, n=30) for the repeated measurements and 0.988 (p<0.001, n=30) for the data between different observers. The mean difference of internal diameter measured was 5.7±1.1 μm (mean±SEM) between the repeated measurements and 8.4±1.3 μm for the different observers.

**Drug Administration**

Drugs were given either topically through the superfusate or intra-arterially through the polyethylene tubing cannulated into the side branch of mesenteric artery. For the intra-arterial infusion of drugs, we used an infusion pump (model A-II, Nakagawa Seikodo, Tokyo, Japan) and minimized the infusion rate to 0.03 ml/min or less. Drugs used were serotonin (Sigma Chemical Co., St. Louis, Mo.), norepinephrine (Sankyo, Tokyo, Japan), acetylcholine (Daiichi Seiyaku, Tokyo, Japan), and CHAPS.
Endothelial Damaging

Endothelial damaging was done by intra-arterial administration of the detergent CHAPS. We dissolved CHAPS in Tyrode's solution at a concentration of 0.3% and infused the solution at a rate of 0.03 ml/min into the vascular lumen for 30–40 seconds; the mesenteric artery had been precontracted by 1.0 μg/ml norepinephrine given into the superfusate so that the vascular lumen could be filled preferentially with the CHAPS solution at a lower infusion rate. This method minimized the total dose of CHAPS administered and, thus, its possible systemic effects. We confirmed endothelial damage by examining the dilator response to intra-arterial infusion of 0.4 μg/min acetylcholine of the mesenteric artery precontracted with 1.0 μg/ml norepinephrine given into the superfusate.

Histological Examination

After the end of the experiments, the mesenteric arteries were removed and placed in 10% formalin. Histological processing was done by conventional methods, and sections were subjected to hematoxylin-eosin staining or to van Gieson staining.

Statistical Analysis

All data are expressed as mean±SEM. The difference in diameter between the upstream and downstream site or between intact and damaged endothelium was examined using Student's t test for paired data. Statistical difference among three groups or more was examined using analysis of variance and modified t test (Bonferroni method).

Results

Effects of Nonocclusive Thrombus Formation on Vascular Tone

The first compression damage of the vascular segment induced constriction of adjacent short segments of both upstream and downstream sites. The center of the damaged site dilated relatively. Vasocostriction at the downstream site disappeared within a few minutes after compression damage, by which time a small thrombus was formed; vasocostriction at the upstream site persisted for 10–15 minutes. Additional compression did not induce such vasoconstriction.

Further increase in thrombus size did not influence the tone of the downstream vascular bed unless the blood flow was interrupted by an occlusive thrombus. The internal diameter approximately 1,000 μm downstream from the thrombus was 295±22 μm (n=7) before thrombus formation and did not change significantly even after luminal narrowing of about 70–90% by the thrombus (293±22 μm, p=NS).

Figure 2 shows representative photographs of the effects of topical serotonin (3.0 μg/ml) on the diameter of the mesenteric artery. Serotonin constricted the mesenteric artery, but the vascular response was much less at the downstream site of the thrombus than at the upstream site (Figure 2B). Vasocostriction was inhibited equally over the entire downstream vascular bed that was observable (i.e., more than 20 mm from the thrombus or down to the boundary between the mesentery and the small intestine). The constrictor response was inhibited even with a smaller thrombus that narrowed the vascular lumen by less than 50%. Serotonin constricted both the upstream and downstream vascular bed when given 60 minutes after compression damage, by which time no thrombus formation was observed (Figure 2C).

Figure 3 summarizes the effects of topical serotonin and norepinephrine on the internal diameter of mesenteric arteries at sites both upstream and downstream from the thrombus. Serotonin decreased the diameter at the upstream site dose dependently, and at a concentration of 30 μg/ml, the diameter was reduced to 45±7% (n=7) of control. At the downstream site, however, constrictor response to seroto-
In experiments, a contraction of the vascular lumen (about 70–90%) not induced by thrombus but by a mechanical compression with a glass micropipette did not inhibit the constrictor response of the downstream site to serotonin or norepinephrine (n=4). A topical application of tetrodotoxin 0.3 μg/ml did not affect the inhibitory action of thrombosis either (n=3). These results indicate that inhibition is induced neither by luminal narrowing itself nor by perivascular nerve activation. Furthermore, an interruption of the flow by a mechanical obstruction at a site far downstream restored the constrictor response of the vascular segment downstream from the thrombus (n=5), indicating that the inhibition is flow dependent.

Figure 4 summarizes endothelium-dependent relaxation of the mesenteric artery induced by acetylcholine and its inhibition by endothelial damaging. The topical application of 1.0 μg/ml norepinephrine through the superfusate reduced the internal diameter markedly from 306±22 to 115±11 μm (n=7, p<0.01). In mesenteric arteries without endothelial damaging, an intra-arterial infusion of 0.4 μg/min acetylcholine fully reversed the vasoconstriction induced by norepinephrine (to 306±20 μm), as shown in the left panel of Figure 4. Endothelial damaging induced by intra-arterial infusion of CHAPS caused mild reduction in the diameter even at control (306±22 μm before and 251±16 after the damaging, n=7, p<0.01). The administration of acetylcholine did not fully dilate the mesenteric artery precontracted with norepinephrine, and the diameter after acetylcholine administration was significantly lower than that at control (132±17 μm, p<0.01 versus control, Figure 4, right panel). The narrowing of the vascular lumen (about 70–90%) not induced by thrombus but by a mechanical compression with a glass micropipette did not inhibit the constrictor response of the downstream site to serotonin or norepinephrine (n=4). A topical application of tetrodotoxin 0.3 μg/ml did not affect the inhibitory action of thrombosis either (n=3). These results indicate that inhibition is induced neither by luminal narrowing itself nor by perivascular nerve activation. Furthermore, an interruption of the flow by a mechanical obstruction at a site far downstream restored the constrictor response of the vascular segment downstream from the thrombus (n=5), indicating that the inhibition is flow dependent.

**Figure 3.** Graphs showing effects of topically applied serotonin and norepinephrine on the internal diameter of mesenteric arteries at sites upstream (closed circles) and downstream (open circles) from the thrombus. Data are mean±SEM from seven experiments. *Significant difference (p<0.01) between upstream and downstream sites.

**Figure 4.** Bar graphs showing effects of endothelial damaging on endothelium-dependent relaxation induced by intra-arterial infusion of 0.4 μg/min acetylcholine (ACh). The mesenteric artery was precontracted with 1.0 μg/ml norepinephrine (NE) given into the superfusate. Data are mean±SEM from seven experiments. NS, no significant difference.
control to 77±21 μm after thrombotic occlusion, n=7, p<0.01). The diameter then increased very slowly even during the thrombotic occlusion. One to 4 minutes after the occlusion, the thrombus began to break down from its peripheral region attached to the vascular wall. After the reflow, the decreased vascular diameter returned to control level quickly. Nonthrombotic mechanical obstructions of the arter-

Figure 5. Graph showing effects of norepinephrine on the internal diameter of mesenteric arteries with damaged endothelium and thrombus formation. Closed and open circles indicate sites upstream and downstream from the thrombus, respectively. Data are mean±SEM from seven experiments.

Figure 6. Representative photographs showing the effects of occlusive thrombus on vascular tone. Panel A: Control. Panel B: Marked diameter reduction at the downstream site after thrombotic luminal occlusion. Panel C: Dilation of the downstream site after topical application of 10 μg/ml acetylcholine under thrombotic occlusion. Thin arrow indicates thrombus, and thick arrow indicates the direction of blood flow. Horizontal bar, 100 μm.

Figure 7. Bar graphs showing reduction in the internal diameter at the site downstream from the thrombus and its reversion by 10 μg/ml topically applied acetylcholine (ACh). Mechanical occlusion indicates nonthrombotic luminal occlusion induced by a mild vascular compression with a glass micropipette. Data are mean±SEM from seven experiments.

ies showed only small reductions in the diameters at the downstream sites (to 241±16 μm, Figure 7), and thus, diameter reductions after thrombotic occlusion may not be caused by passive collapse due to decreased intravascular pressure but by active constriction of the artery.

Topical application of acetylcholine (10 μg/ml) dilated the downstream vascular bed, which had been constricted after the thrombotic occlusion. The dilation developed even during the luminal occlusion by the thrombus, as shown in Figure 6C. The diameter increased significantly from 77±21 μm to 270±31 μm (n=7, p<0.01), which was almost comparable with the control value (Figure 7).

Discussion

In the present study, we demonstrated that thrombus formation did not constrict the downstream vascular bed but inhibited its constrictor response to both serotonin and norepinephrine as long as the blood flow was not interrupted by the thrombus. These anticonstricting effects of thrombosis were no longer observed after damage to the vascular endothelium. However, when the thrombus enlarged and occluded the vascular lumen, strong vasoconstriction developed at the site downstream from the thrombus even in vessels having intact endothelium. To our knowledge, such significant dual effects of thrombosis on vascular tone have never been demonstrated in vivo.

The inhibition of vasoconstrictor response observed at the site downstream from thrombus was not caused by accidental damage to the site during the preparation, because the constrictor response of the site to serotonin was restored later when thrombus formation
was no longer observed. Neither luminal stenosis per se, caused by thrombus and subsequent changes in flow-mediated shear stress, nor intraluminal oxygen tension contributed to the inhibition of vasoconstriction, because mechanical luminal stenosis not induced by thrombus had no such inhibitory effects. After the mechanical interruption of the flow through the thrombus to the downstream site, the anticonstricting effect disappeared. These results suggest that the inhibition of constriction is induced by some active substance(s), probably with short plasma half-life, flowing down from the thrombus.

Endothelial damaging was done by an intraarterial infusion of a detergent as reported by Randell and Hiley. Damage to the endothelium was confirmed by a significant inhibition of endothelium-dependent dilation induced by intra-arterial infusion of acetylcholine. In these mesenteric arteries with damaged endothelium, thrombosis did not exert anticonstricting effects on the downstream vascular bed, as evidenced by a similar constrictor response to norepinephrine at the upstream and downstream sites. This will suggest that some substance(s) released during thrombus formation causes endothelium-dependent inhibition of constriction of the downstream vascular bed. To our knowledge, this is the first report to demonstrate in vivo the chain of events from the partially occlusive thrombus to the endothelium.

During thrombus formation, many vasoactive substances are released from aggregating platelets and leukocytes, as well as from the blood coagulation process. At present, it is not clear which substance(s) released during thrombus formation is responsible for the endothelium-dependent inhibition of vasoconstriction. In studies in vitro with coronary ring preparations, adenosine diphosphate is reported to be a causative substance for the endothelium-dependent dilator response to aggregating platelets in both dogs and humans. We need further evaluation to determine whether anticonstricting effects of thrombosis observed in the present study in vivo are also caused by adenosine diphosphate and whether this phenomenon is comparable with that observed in previous studies in vitro.

Although vasoconstriction has long been thought to develop during thrombosis or platelet aggregation, evidence for such vasoconstriction has just recently been provided in animal models in vivo. In porcine carotid arteries, Lam et al. observed constriction of short segments just proximal and distal to vessel wall damage induced by balloon angioplasty and found a positive correlation between the vasoconstrictive response and platelet deposition. In the constricted segments, the endothelium was found denuded. Severe mechanical constriction of canine coronary artery induces cyclic flow reduction, which is caused by platelet deposition on the stenosed segment with endothelial damage. In this model, Golino et al. reported constriction of the distal coronary segment with endothelial damage during the flow reduction caused by platelet deposition.

In the present study, the vasoconstriction after thrombotic occlusion was observed in mesenteric arteries even without endothelial damaging. Accidental damage to the endothelium during preparation was unlikely, because the arterial segment had intact endothelium-dependent relaxant response to acetylcholine and because the histological examination revealed an intact endothelial lining. Thus, our results seem different from those of previous reports in regard to the intact endothelium and appear to be the first such observations.

The underlying mechanism for the vasoconstriction of arteries with endothelium developing after thrombotic luminal occlusion remains to be elucidated. However, the cessation of flow or flow-mediated shear stress per se or subsequent hypoxia were not the cause of the vasoconstriction, because nonthrombotic mechanical occlusion did not constrict the downstream vascular bed significantly. The vasoconstriction was always accompanied by intraluminal debris of the thrombus flowing down slowly just before the thrombotic occlusion. This will strongly suggest that the vasoconstriction is caused by some substances released from the thrombus.

Most substances released from aggregating platelets and leukocytes, as well as thrombin delivered during the activation of the blood coagulation system, may exert not constrictor but dilator actions in arteries with endothelium. The present results with nonocclusive thrombosis have strongly suggested that this is also the case in vivo. It could be speculated that the cessation of flow inhibited such endothelium-dependent relaxation, and thus, the direct constrictor effects on vascular smooth muscle dominated. However, endothelium-dependent relaxation seems not to be inhibited, at least generally, because acetylcholine fully reversed the vasoconstriction even during the cessation of flow.

Short plasma half-life of substances stimulating endothelium and exerting an anticonstricting effect may be a plausible explanation for the vasoconstriction after thrombotic occlusion, which may be caused by some other substances with longer plasma half-life and direct constrictor effects on vascular smooth muscles. This is very likely if adenosine diphosphate is a causative substance for the endothelium-dependent anticonstricting effect of thrombosis in vivo, as already reported in studies in vivo. Because these substances are metabolized quickly by ectoenzymes located at the endothelium and disappear from the blood without further supply from the thrombus.

Production of substances that can constrict mesenteric arteries even with intact endothelium or an increase in those substances in the plasma may be a possible mechanism for vasoconstriction after thrombotic occlusion. Thromboxane A₂, a potent vasoconstrictor substance, seems not to possess endothelium-dependent dilator action and could be a mediator of
vasoconstriction after thrombotic occlusion. The activation of the blood coagulation system is a distinct feature occurring after the cessation of blood flow. It can be speculated that some vasoconstrictor substances other than thrombin are produced during the activation of the blood coagulation system and are the cause of constriction of mesenteric arteries. Another possible vasoconstrictor substance may be endothelin, which is released from the endothelium by stimulation with thrombin. However, rat endothelin is reported to possess vasodilator action in mesenteric arteries. In future studies, causative substances for vasoconstriction should be identified.

The present finding will have important pathophysiological implications. The anticonstricting effects of thrombosis on the downstream vascular bed may be beneficial in the preservation of blood flow to tissue in the face of thrombotic luminal narrowing. The strong vasoconstriction after the thrombotic occlusion of vascular lumen may primarily work as a self-defense mechanism for hemostasis.

References


Key Words • antivasoconstrictor • vasoconstriction • endothelium • thrombosis
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