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The Effects of Thrombus, Thrombectomy and Thrombolysis on Endothelial Function

T. D. Reil, W. S. Moore*, V. S. Kashyap, S. S. Nene, H. A. Gelabert and W. J. Quinones-Baldrich

Division of Vascular Surgery, University of California Los Angeles, UCLA Center for Health Sciences, Gonda (Goldschmied) Vascular Center, Los Angeles, CA, U.S.A.

Objective: *this study was undertaken to examine and compare the effects of thrombus, thrombectomy, and thrombolysis on endothelial function as measured by endothelium-dependent vasorelaxation (EDR).*
Methods: *adult, male New Zealand white rabbits underwent ligation of the left common iliac to femoral artery to induce thrombosis and were then randomly assigned to one of five groups, n = 6 in each. Group A consisted of ligation and thrombosis for 4 h. Group B underwent similar ligation for 4 h, but without intraluminal thrombus present. Following 4 h of ligation and thrombosis, Group C underwent thrombectomy while group D was treated with urokinase (UK), 4000 U/min for 30 min. Group E underwent UK infusion alone. The right external iliac artery served as control vessel in each group. All arteries were removed and endothelial function was determined by measuring EDR.*
Results: *the presence of thrombus reduced EDR by 50% (group A) compared to control. Vessels with interrupted flow, but not exposed to thrombus, retained normal EDR (group B). Thrombectomy decreased EDR significantly (group C) compared to thrombolysis (group D) and control. UK did not significantly alter EDR (groups D, E).*
Conclusions: *exposure of endothelium to thrombus significantly decreases EDR. EDR was not affected by interruption of blood flow in the absence of thrombus. Thrombectomy appeared to cause a further additive insult to the endothelium. In contrast, thrombolysis with UK preserved residual endothelial function. These data suggest that it is important to differentiate the effects of thrombus on endothelium from effects due to thrombectomy or thrombolysis when evaluating treatment modalities for arterial thrombosis.*

Key Words: Endothelial function; Thrombus; Endothelial-dependent relaxation; Thrombectomy; Thrombolysis; Arterial thrombosis.

Introduction

The treatment of acute arterial occlusion due to thrombosis is an area of continued investigative and clinical study in vascular surgery. Multiple experimental and clinical studies have compared the treatment options for managing acute arterial thrombosis, including traditional surgical thrombectomy and the use of catheter-directed thrombolysis.[1–4] While clinical data comparing the two treatment modalities accrue, there remains a limited amount of experimental data on their effects on vessel wall physiology. Previous work examining the effects of thrombectomy and thrombolysis on endothelial physiology has suggested thrombolysis to be superior to thrombectomy, with lytic therapy appearing to be less damaging to endothelial function.[5,6] However, these studies fail to separate effects on endothelium-dependent relaxation (EDR) induced by exposure of the intima to thrombus from subsequent effects on endothelial function due to any interventions performed. This distinction may be important when comparing the effect of either treatment modality on vascular wall physiology. If thrombus has altered the endothelium prior to an intervention, the subsequent effects on endothelium attributed to an intervention may not be reflected correctly in the final analysis.

The vascular endothelium plays a significant regulatory role in many physiological processes in the body, including the regulation of vasomotor tone. The endothelial cell produces substances that modulate vascular tone, the most important of which is endothelium-dependent relaxing factor (EDRF), also known as nitric oxide.[8–10] Nitric oxide released from endothelium diffuses to underlying smooth-muscle cells to induce vasorelaxation. Experimental models using arterial segments with intact, functioning endothelium will vasodilate when exposed to agents known to

* Please address all correspondence to: W. S. Moore, Room 72-215, Department of Surgery, Division of Vascular Surgery, UCLA Medical Center, 10833 LeConte Avenue, Los Angeles, CA, 90095-6904, U.S.A.

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induce nitric oxide release (endothelium-dependent relaxation). By examining the relaxation responses, one can assess endothelial viability and function.

In this experiment, we set out first to define the effects of thrombus on endothelial function by measuring EDR. We then compared the effect of thrombectomy and thrombolysis on EDR. We hypothesised that endothelial function is decreased by acute exposure to thrombus. Furthermore, any additive effects of thrombectomy and thrombolysis on endothelial function after thrombosis was assessed. In comparing the effects of each treatment modality, we attempted to differentiate changes in EDR due to thrombus from those due to subsequent interventions.

Materials and Methods

Animals and procedures

Thirty adult, male New Zealand white rabbits (Charles River Breeding Laboratories, Oakland, CA, U.S.A.) weighing 2–3 kg were divided into five groups. All rabbits were housed in standard cages and allowed free access to standard rabbit chow and water *ad libitum*. All animals were handled and cared for under institutional guidelines in compliance with "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals" (NIH no. 86-23, revised 1985). The rabbits were anaesthetised by intramuscular injection of ketamine (100 mg/kg) and acepromazine (5 mg/kg). After adequate anaesthesia, the abdomen was shaved and an inferior midline abdominal incision was made. The abdominal aorta and bilateral iliac arteries were identified and isolated with elastic vessel loops. In all groups, the right external iliac artery served as a non-operated control and the left external iliac artery served as the experimental vessel. The left internal iliac artery and all side branches to the external iliac artery were ligated with small surgical clips. The animals were then randomly assigned to one of five experimental groups.

Group A consisted of ligation and thrombosis. Medium surgical clips were placed on the left common iliac artery to occlude inflow and on the distal external iliac artery at the inguinal ligament to occlude backflow. The clips were left in place for 4 h. A hand-held Doppler probe was used to confirm lack of blood flow through the occluded segment. The presence of thrombus in the arterial segment was confirmed visually at the time the artery was removed for ring studies. Group B underwent similar ligation for 4 h, but without intraluminal thrombus present. In this group, after the same clips were placed, a small arteriotomy was made in the distal external iliac artery. The artery was gently flushed with 3 cc of normal saline using a 22-gauge angiocatheter placed through the arteriotomy to remove all residual blood. The vessel remained ligated for 4 h. It should be noted that the segment of artery used for ring studies was proximal to the arteriotomy site. In a second set of experiments, following 4 h of ligation and thrombosis, group C underwent thrombectomy. The thrombectomy was performed by making an arteriotomy in the left femoral artery and inserting a 2-French Fogarty embolectomy catheter (Edwards Laboratories, Puerto Rico). The catheter was carefully advanced proximally into the thrombosed left external iliac artery. The balloon was then inflated to 5 PSI measured with an attached pressure gauge (Nameic, New York, NY, U.S.A.) and slowly withdrawn to evacuate the thrombus. 5 PSI was chosen based on pilot studies indicating that this pressure within the balloon catheter did not cause gross endothelial damage, by microscopic studies. Only one passage of the balloon catheter was performed for each animal. Following 4 h of ligation and thrombosis, group D was treated with urokinase (UK) infusion (Abbott Laboratories, Chicago, IL, U.S.A.). A 25-gauge butterfly needle was placed into a side branch at the junction of the external and internal iliac arteries and secured in place with 4-0 silk suture. This was connected to an infusion pump (Harvard Apparatus, Holliston, MA, U.S.A.) and UK was infused at a rate of 4000 U/min for 3 min. As the infusion was started, the distal clip was removed from the external iliac artery. Group E, serving as UK control, received the same infusion of UK, but without prior ligation or thrombosis.

After the above interventions, both external iliac arteries were removed and prepared for measurement of endothelial function using arterial rings in an organ chamber.

Studies of endothelial and smooth-muscle-cell function

The external iliac arteries were carefully removed and placed in chilled Krebs–Henseleit physiological solution (NaCl 118 mM, $NaHCO_3$ 25 mM, glucose 5.6 mM, KH_2PO_4 1.2 mM, KCl 4.7 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 1.5 mM). All remaining fat and connective tissue was gently removed and each artery was sectioned into 5-mm segments. The segments were mounted on standard tungsten wire triangles (A-M Systems, Everett, WA, U.S.A.), attached to isometric force displacement transducers (FTO3C, Grass Instrument Co., Quincy,

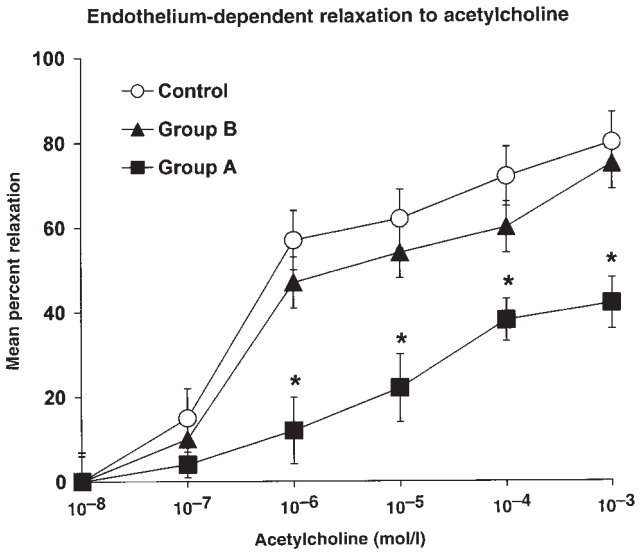


Fig. 1. Endothelium-dependent relaxation (EDR) response to acetylcholine. Exposure of endothelium to thrombus for 4 hours (group A) significantly decreases vasorelaxation of the arterial segment compared to control and compared to lack of flow without thrombus present (group B). Arterial segments were first contracted to 75% F-max with 10^{-5} mol/l phenylephrine. Relaxation was then induced using increasing doses of acetylcholine. Relaxation is expressed as mean percent relaxation (±standard error) in response to incremental doses of acetylcholine. The means of each group at the various doses of acetylcholine were compared via ANOVA, $n=6$ in each group. (*$p=0.05$).

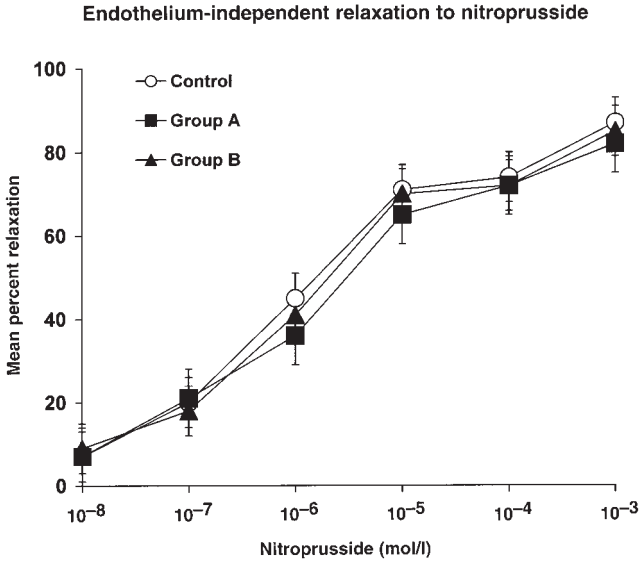


Fig. 2. Endothelium-independent relaxation response to sodium nitroprusside. All vessels relaxed uniformly to nitroprusside in a concentration-dependent fashion. After contraction to 75% F-max with 10^{-5} mol/l phenylephrine, relaxation was induced with increasing concentrations of sodium nitroprusside. Results are expressed as mean percent relaxation (±standard error). Groups were compared via ANOVA. There were no significant differences between the groups, $n=6$ in each group.

MA, U.S.A.), and placed into tissue baths. The transducer output was amplified and recorded continuously on a portable computer with digital analysis software (FemtoTek, Inc, Mt Laurel, NJ, U.S.A.). The tissue baths were temperature-controlled via a heated-water jacket at 37 °C. Standard Krebs–Henseleit solution was used with a 95% oxygen, 5% carbon dioxide gas mixture bubbled into the baths.

Based on previous studies, pre-load (2 g) was applied to the arterial rings and the vessels were allowed to equilibrate for 45 min. After equilibration, the arteries were constricted using a high-potassium Krebs' solution (standard Krebs' but with 122 mM potassium chloride) and allowed to re-equilibrate. This represented the maximal contractile force for the artery (F-max). The baths were then emptied and rinsed three times with Krebs' solution and again allowed to equilibrate. The arteries were then constricted to 75% of F-max with 10^{-5} M phenylephrine (Sigma, St. Louis, MO, U.S.A.). Acetylcholine (Sigma, St. Louis, MO, U.S.A.) was then added in incremental log concentrations from 10^{-8} M to 10^{-3} M for determination of EDR. EDR was also measured in response to bradykinin and calcium ionophore A23187 added in incremental log concentrations from 10^{-8} M to 10^{-3} M. Endothelium-independent relaxation was measured in

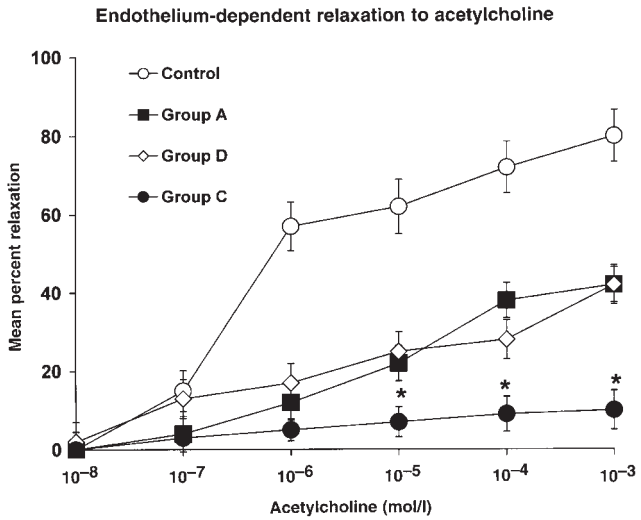


Fig. 3. Endothelium-dependent relaxation (EDR) response to acetylcholine. Thrombectomy significantly reduces EDR (group C) compared to urokinase (group D) and control. Although the UK-treated group had decreased EDR compared to control, the decrease was not additive to the effects attributable to thrombus. Arterial segments were contracted to 75% F-max with 10^{-5} mol/l phenylephrine. Relaxation was then induced using increasing doses of acetylcholine. Relaxation is expressed as mean percent relaxation (±standard error). The means of each group at various dosages were compared via ANOVA, $n=6$ in each group. (*$p<0.05$).

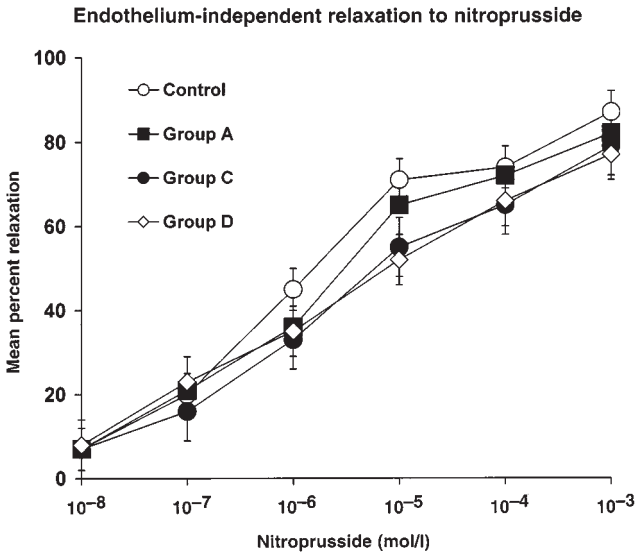


Fig. 4. Endothelium-independent relaxation response to sodium nitroprusside. There are no differences in relaxation between the groups, indicating no effect on smooth-muscle function by thrombus, thrombectomy, or thrombolysis. After contraction to 75% F-max with 10^{-5} mol/l phenylephrine, relaxation was induced with increasing concentrations of sodium nitroprusside. Results are expressed as mean percent relaxation (±standard error). Groups were compared via ANOVA. There were no significant differences between the groups, $n=6$ in each group.

a similar fashion using increasing incremental log concentrations of sodium nitroprusside (all reagents from Sigma). Baths were rinsed and arterial segments were brought to 75% F-max with phenylephrine between each reagent.

Tension was measured in grams and contraction and relaxation were recorded as percentages of 75% F-max for each incremental dose of reagent. Contraction of arteries to high-potassium Kreb's solution and phenylephrine were recorded in grams of tension developed.

Statistical analysis

Relaxation responses were recorded as percentages of 75% F-max at each dose of acetylcholine, bradykinin, and calcium ionophore. The mean value at each concentration for each group was determined. Standard error of the mean was also determined. The means were compared for significant differences by a one-way analysis of variance (ANOVA) and *post hoc* comparisons for differences between group pairs. Contraction responses to high potassium and PE were recorded in grams of tension developed, means were calculated at each dose, and the data again compared by ANOVA.

Scanning electron microscopy

Arterial segments were taken from all five groups and prepared for scanning electron microscopy as described previously.[11] One artery from each treatment group was examined. Photographs were taken at multiple magnifications from five random areas of each artery. The micrographs were examined for any obvious gross alterations in the endothelium or areas of endothelial denudation.

Results

Endothelium-dependent relaxation

After contraction to 75% F-max with phenylephrine (10^{-5} M), exposure of arteries to acetylcholine, bradykinin, and calcium ionophore resulted in concentration-dependent vasorelaxation. At maximal acetylcholine concentration (10^{-3} M), group A relaxed 41% (±6.2%) while controls relaxed 80% (±5.1%) (Fig. 1). The controls relaxed 50% more than group A ($p<0.05$). Significant differences ($p<0.05$) in percent-relaxation were also noted at 10^{-4}, 10^{-5}, and 10^{-6} M acetylcholine (Fig. 1). Group B relaxed 75% (±7.0%) to acetylcholine (10^{-3} M). There was no difference between group B compared to control ($p>0.10$). Similar results were obtained with bradykinin and calcium ionophore.

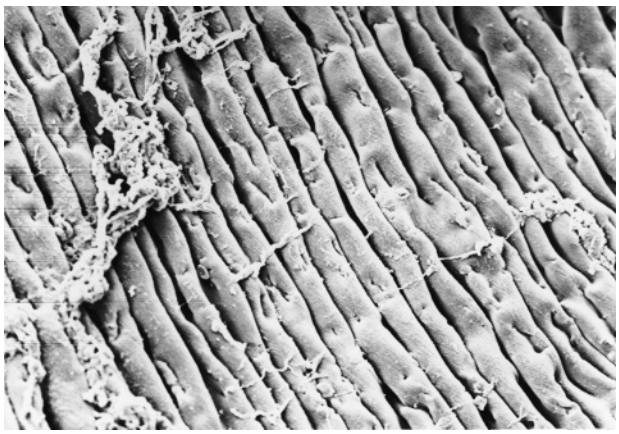
At maximal acetylcholine concentration (10^{-3} M), group C only relaxed 10% (±3.9%) while group D relaxed 48% (±6.2%) (Fig. 2). Relaxation in group C was significantly depressed compared to control ($p<0.05$). Relaxation in group C was significantly less than group D ($p<0.05$). No statistical difference was noted between groups A and D. Similar results were obtained with bradykinin and calcium ionophore.

EDR in group E was not altered compared to control at any dose. Group E relaxed 77% (±6.0%) at maximal acetylcholine concentration (10^{-3} M).

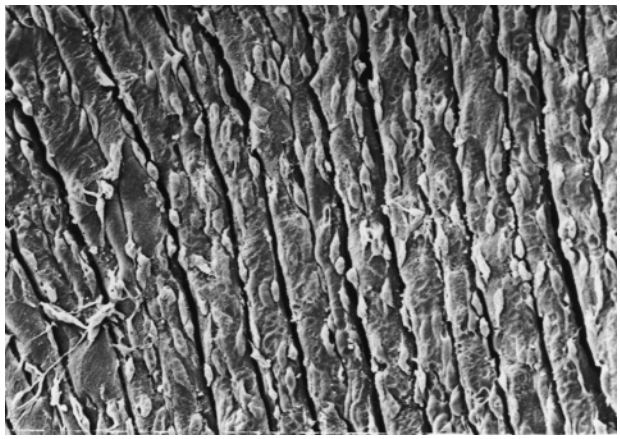
Endothelium-independent relaxation

All arterial ring segments relaxed uniformly to sodium nitroprusside in a concentration-dependent fashion. At maximal sodium nitroprusside concentration (10^{-3} M), group A relaxed 85% (±8.0%), group B 87% (±6.2%), and control 88% (±4.9%). No significant differences were noted between the three groups (Fig. 3).

At maximal sodium nitroprusside concentration



(A)



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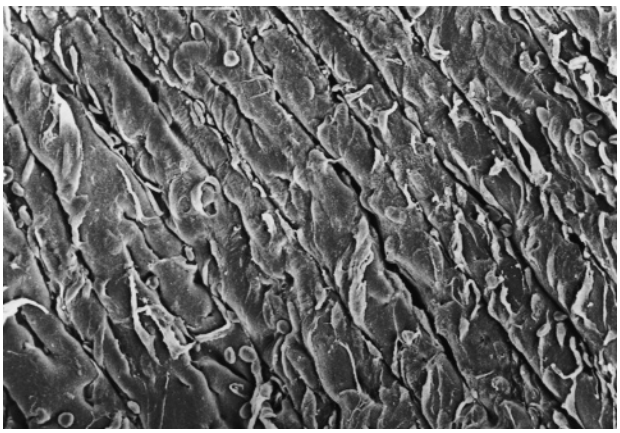

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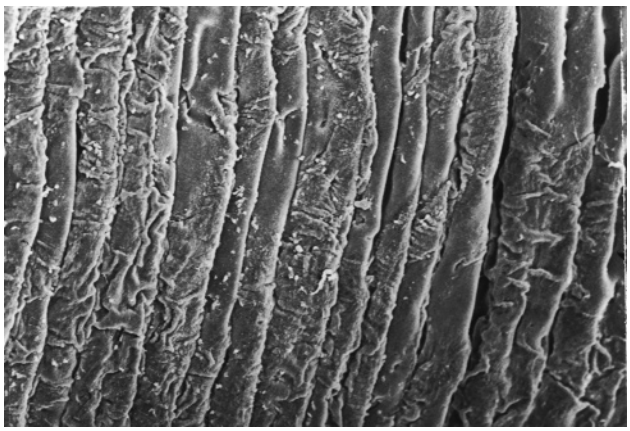
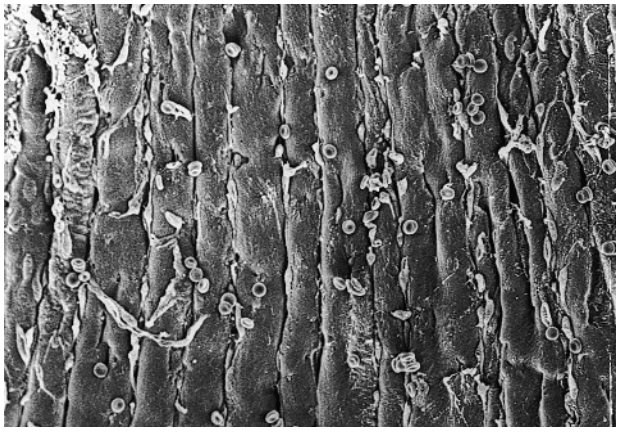


(D)



(E)

Fig. 5. (A–E) Scanning electron microscopy (SEM) of arterial segments. SEM was performed on arteries from each group after vessels were harvested for ring studies. SEM photos were taken of random areas of vessels to compare endothelial morphology and intimal surface. Gross inspection indicated no significant differences between the intima and endothelium in any of the groups. An intact endothelial monolayer appears in all groups. (5A = control, 5B = lack of flow with intraluminal thrombus present, 5C = lack of flow without thrombus present, 5D = thrombectomy after thrombus and no flow, 5E = urokinase after thrombus and no flow).

(10^{-3} M), group C relaxed 86% ($\pm$7.6%), group D 84% ($\pm$5.2%), and group E 86% ($\pm$7.1%). No statistically significant differences between groups were noted (Fig. 4).

Smooth-muscle contraction

The constriction of vessels in response to high potassium Krebs' solution and to phenylephrine (10^{-5} M) was similar in all groups. Mean contraction to high K^+ Krebs' was 1.4 g (group A), 1.4 g (group B), 1.2 g (group C), 1.3 g (group D), 1.4 g (group E), and 1.3 g (control). For phenylephrine, mean contraction was 1.1 g (group A), 0.9 g (group B), 1.0 g (group C), 1.0 g (group D), 1.0 g (group E), and 1.1 g (control). There were no significant differences between groups.

Scanning electron microscopy (SEM)

SEM of arterial segments revealed an intact endothelial monolayer in all groups (Fig. 5). There was no evidence of any intimal denudation or gross changes in endothelial morphology to explain the functional physiological changes observed in the ring studies.

Discussion

The treatment of acute arterial thrombosis remains a difficult clinical entity to manage, in spite of multiple therapeutic advances. The introduction of balloon thrombectomy in 1963 allowed the surgeon a minimally invasive and effective treatment option for thrombectomy compared to previous methods of thrombus extraction.[12] A second and more recent advance was the development of catheter-directed thrombolytic therapy.[13–15] Urokinase thrombolysis has been shown to be a safe, effective method for the clinical management of both native artery and graft thromboses.[14,15] Multiple clinical trials have compared thrombectomy and thrombolysis.[16–18] However, there is very little experimental data upon which to compare the effect of these two modalities on arterial function. In this study, our intent was to compare thrombectomy with thrombolysis with respect to preservation of the biochemical properties of endothelium, by measuring endothelium-dependent relaxation (EDR). Before proceeding with a direct comparison, it was important to determine whether or not the presence of thrombus was deleterious to EDR. Previous publications have demonstrated that various components of thrombus can cause damage to endothelium in tissue culture,[20–23] but little is known about the effects of thrombus on endothelium *in vivo* or specifically on the production of nitric oxide. We were able to demonstrate, in the present study, that exposure of endothelium to thrombus clearly led to a significant decrease in endothelium-dependent vasorelaxation, indicating altered endothelial function. This effect appears to be independent of cessation of luminal blood flow, suggesting that some component of thrombus may be damaging to the endothelium. SEM of the arteries failed to demonstrate significant alterations in endothelial morphology due to thrombus, suggesting that the decrease in EDR may be due to an alteration in the endothelial cell physiology. This altered vasorelaxation appears to be due solely to changes in endothelium, as thrombus did not alter endothelium-independent relaxation (smooth-muscle function), as shown by uniform relaxation of all vessels to nitroprusside. Furthermore, smooth-muscle-cell contractility was not affected by the thrombus, demonstrated by a normal contractile response to potassium and phenylephrine.

Having demonstrated that thrombus itself has an adverse effect on EDR, we were able to proceed with the second portion of the study; a direct comparison of the effects of thrombectomy versus thrombolysis on endothelium-dependent relaxation. Previous work suggested that urokinase was superior to thrombectomy for treating acute arterial thrombosis,[5,6] while thrombectomy was shown to significantly reduce EDR and appeared to cause gross changes in endothelial morphology. In both studies, urokinase appeared to preserve endothelial structure and function. In our present study, it was clearly shown that thrombectomy significantly reduced EDR compared to thrombolysis. Thrombectomy had an additive decrease in EDRF compared to the effects of thrombus alone. However, SEM did not demonstrate any morphological changes in endothelium, nor did they show evidence of denudation of endothelium by thrombectomy. While we acknowledge that drawing conclusions from one artery per group may not reflect the actual result in all the artery segments from the experiment, the presence of endothelium in each artery studied by SEM suggests that endothelial loss is not a likely explanation for the experimental findings. Thrombectomy appears to have an additive detrimental effect on endothelial function that is not attributable to gross changes in endothelial morphology. In contrast, urokinase thrombolysis preserved endothelium-dependent relaxation. Although the urokinase-treated group had decreased EDR compared to controls, there was no difference between the urokinase-treated group and the group exposed to

thrombus, indicating that urokinase did not have an additive detrimental effect that could not already be attributed to thrombus. Assuming that endothelium exposed to thrombus already has a reduced EDR, thrombolysis with urokinase appeared to preserve residual EDR. This finding emphasises the importance of examining the effect of thrombus alone on endothelium. Without prior observation, one would be tempted to conclude that thrombolysis is damaging to endothelium when, in fact, it preserved endothelial function.

From these studies we conclude that thrombus is damaging to endothelium as measured by altered EDR. This effect on endothelium is the same for multiple agonists, including acetylcholine, bradykinin, and calcium ionophore. The uniform relaxation in response to sodium nitroprusside, as well as uniform contraction in response to phenylephrine and potassium, suggests that short-term thrombus does not affect smooth-muscle function. Thrombectomy, in contrast, acutely produced an additive decrease in EDR, while thrombolysis preserved residual EDR. In the acute timeframe, thrombolysis with urokinase appears to be better tolerated by endothelium, but prior damage to the endothelium by the presence of thrombus may explain varied results seen clinically. However, one cannot conclude that urokinase is ultimately preferential to thrombectomy until long-term comparisons of each modality's effect on endothelium are performed. This will be the subject of future experiments.

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