SEM evaluation of endothelial damage following temporary middle cerebral artery occlusion in dogs

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Microsurgical clips and tourniquets were used to occlude middle cerebral arteries of dogs for 45-minute periods. Scanning electron microscopy and light microscopy studies revealed significant endothelial damage in many of these arteries. Less traumatic microsurgical clips are needed for temporary small vessel occlusion.

KEY WORDS □ microvascular surgery □ scanning electron microscopy □ endothelial damage □ vascular clip

The commercially available microclips currently used for temporary occlusion of small arteries are simply miniature versions of cerebral aneurysm clips. They were originally designed for permanent placement, and inflict considerable endothelial damage when applied temporarily to small arteries.

Materials and Methods

This study included 150 mongrel dogs weighing between 15 and 25 kg, divided into acute and chronic groups. The acute animals were divided into six subgroups of 20 each, while the chronic animals were divided into six subgroups of five each.

Each animal was anesthetized with intravenous pentobarbital (25 mg/kg), intubated, and placed on a Harvard respirator.*

Supplemental doses of pentobarbital were added in order to maintain a good level of surgical anesthesia. Arterial blood gases were checked 20 minutes after intubation, and the respirator was adjusted accordingly to maintain the pCO₂ between 35 and 45 torr and the pH around 7.40. A femoral arterial catheter was inserted and connected to a Statham transducer† and Grass polygraph for blood pressure (BP) monitoring.‡ Electrocardiograph findings and temperature were also recorded.

The right side of the head was then shaved, aseptically prepared, and draped in a sterile manner. Following a right temporal craniotomy, the operating microscope was

*Respirator manufactured by Harvard Apparatus, Inc., 150 Dover Road, Millis, Massachusetts.
†Transducer manufactured by Statham Instruments, 2230 Statham Boulevard, Oxnard, California.
‡Polygraph manufactured by Grass Instrument Company, 101 Old Colony Avenue, Quincy, Massachusetts.

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brought into focus. The dura was opened and the proximal right middle cerebral artery (MCA) was identified. The arachnoid over the proximal MCA was opened and gently dissected away from the artery for a distance of 5 mm from its origin. A 2-mm section of MCA trunk was then selected and completely dissected with a fine Jannetta nerve hook. A micro-occluder was then applied to the right MCA trunk for a period of 45 minutes. In all of the animals, the left MCA was the control, and underwent the same histological preparation as the experimental right MCA.

Each of the six experimental groups was treated with a different micro-occluding device. The distribution was as follows:

Subgroup A: Mayfield MMI clip
Subgroup B: Khodadad clip
Subgroup C: Heifetz clip
Subgroup D: Micro Scoville-Lewis clips
Subgroup E: Microtourniquet
Subgroup F: Microblock

In the acute study, blood flow was restored for 20 minutes following the removal of the micro-occluder, before the recovery of the specimens. In the chronic study, the micro-occluder was removed and the wound was closed in layers. The specimens were recovered 1 month later.

All of the animals were killed with KCl injections, and each brain was carefully removed in toto. The MCA's of both the control and experimental groups were then excised under the operative microscope using ×25 magnification, opened longitudinally, and gently washed with normal saline solution. The flattened artery was fixed with buffered 2% glutaraldehyde (pH 7.2) solution for 5 minutes and then stored in the same solution for 24 hours. The specimen was dehydrated by serial passage through 30%, 50%, 75%, and 95% ethanol. Preparation for scanning electron microscopy included critical-point drying with CO₂ for complete dehydration of the specimen. Each specimen was then mounted on a circular aluminum stud 1 cm in diameter and coated with gold palladium 100 to 400Å thick in a vacuum chamber. The endothelial surface was then examined at ×40 to 16,000 by scanning electron microscopy (SEM). All photographs were made with a Polaroid camera. The photographs were then enlarged to 5 × 8-in. size, thereby permitting a better assessment of details.

All of the specimens were later rehydrated and prepared for light microscopy according to the technique described by Leffingwell. These sections were stained by hematoxylin and eosin.

Results

The 150 normal, unmanipulated left MCA's were compared with the experimental arteries. Normal arterial endothelium had a constant architecture of fine longitudinal ridges and folds. These folds were about 15 μ across, and their smooth undulations comprised a "normal" pattern (Fig. 1). Neither adherent platelet nor fibrin deposits were seen.

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Temporary occlusion by Khodadad clips showed a fairly complete and sharply defined transverse division of the normal endothelial folds in 18 MCA's (Fig. 3). Moreover, small platelet-fibrin deposits were noted on the sections of the media exposed by the disruption
FIG. 2. Endothelium from Subgroup A treated with Mayfield MMI clip, showing laceration and obliteration of the endothelium ridge (DL), and a heavy deposit of fibrin, platelets (P), and red blood cells. N = normal endothelial folds. × 460.

FIG. 4. Endothelium from Subgroup C treated with Heifetz clip, showing disruption (DL) and flattening (F). N = normal endothelial folds. × 270.

FIG. 3. Endothelium from Subgroup B treated with Khodadad clip, showing a sharply transverse section of disruption and laceration of the endothelium folds (DL) with deposit of fibrin and platelets (P). N = normal endothelial folds. × 330.

and retraction of the endothelial margins. Two of the MCA's revealed only flattening of the endothelial folds, and no actual intimal defects were seen.

All 20 arteries occluded by Heifetz clips revealed endothelial surface breaks directly under the clip edges. Very small platelet-fibrin deposits were present in areas of maximal endothelial flattening that corresponded to the apex of the clip's surface curvature (Fig. 4).

Occlusion by small Scoville-Lewis clips revealed a distinctive pattern of endothelial disruption in all 20 arteries (Fig. 5). In each artery, there were paired transverse endothelial cuts about 50 μ across that corresponded to the edges of the clip. The endothelial gaps did not separate much, however, and the intervening endothelium appeared fairly normal. Platelet-fibrin deposits were present along these narrow endothelial defects, but were not as extensive as those noted in the Mayfield and Khodadad clip applications.

Microtourniquet applications produced not only endothelial disruption, but actual retraction of the divided endothelial margins in all the arteries (Fig. 6). This endothelial
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"trough," with exposed media at its base, corresponded with the position of the 6-0 silk suture, which had been cinched down. However, the endothelial edges did not retract as far as in the clip-produced lacerations. In addition, only one intimal tear was present in each artery, so that less media was exposed than in arteries occluded with metal clips.

All 20 Subgroup F arteries occluded by the microblock revealed a distinctive pattern of endothelium flattening and some deposition of fibrin, platelets, and red blood cells (Fig. 7).

In the chronic study, the region of destruction never returned to the original endothelial pattern. The endothelium lost its smooth undulations and became planar. A fine fibrin film covered the region and replaced the endothelium. In many instances, the cellular junctions were widened and clearly observed. Also, fibrin deposits were still seen in this area. This condition was present in 25 chronic study animals. In the five animals with occlusion by microblock, the damage was limited to the endothelial pattern with flattening of the undulations, and there was no fracture of the endothelial layer.
Following the removal of the microtourniquets and microclips, we noted the frequent occurrence of localized arterial dilatation or "ballooning." Examination of these dilated MCA segments by light microscopy revealed a marked separation of the smooth-muscle fibers and diapedesis by red cells (Fig. 8).

**Discussion**

Assessment by SEM and light microscopy has revealed that the commercially available microclips used for temporary arterial occlusion produce significant endothelial damage accompanied often by platelet-fibrin deposits that could jeopardize the results of even the best surgical techniques. Because of the far-reaching consequences of this study, we performed a large number of experiments with each micro-occluder in order to attain statistical certainty.

In the course of examining the endothelium, we were able to differentiate between four degrees of endothelial damage:

**Fig. 8.** Light microscopy of the middle cerebral artery following application of Scoville-Lewis clip. H & E. A: Endothelial ballooning. X 12. B: Destruction of the media layer with diapedesis of the erythrocytes. X 50.

**Fig. 9.** Diagrams and graph showing force (F) necessary to close vessels of various sizes. BP = blood pressure. A = area over which the pressure acts, \(d\) = vessel diameter (a), and \(w\) = clamp width (b). 

\[ F = BP \times A \]

The width of a flattened vessel (c) is essentially one-half of the circumference, \((3.14)(0.5)d\), so the area over which the pressure acts (d) is equal to: 

\[ A = (3.14)(0.5)d \times w \]

Therefore, 

\[ F = (3.14)(0.5)BP \times d \times w = (1.57)BP \times d \times w \]

Where blood pressure is recorded in gm/sq cm and force is reported in grams. If blood pressure is recorded in torr, then 1 mm Hg = 1.36 gm/sq cm, and 

\[ F = (1.36)(1.57)BP \times d \times w = (2.14)BP \times d \times w \]

The graph (right) depicts the forces recessing to close the vessel (F) with a clip width of 0.15 cm for vessels of varying diameters (d) at two different levels of blood pressure (BP). 

\[ F = (2.14)BP \times d \times (0.15) = (0.321)BP \times d \]
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1) flattening, 2) disruption, 3) disruption with clot formation, and 4) fracture, with exposure of the media. Obviously, the lower degrees of damage are preferable, and there are several factors which may be involved in the development of the intimal and internal wall lesions. It is quite likely that the pressure exerted by the clips, tourniquets, and microblocks is excessive. Under ideal conditions, mathematical calculations of the force necessary to close an artery 1 mm in diameter at systolic blood pressure (BP) of 100 mm Hg indicate that a 3-gm force will suffice for a clip 1.5 mm wide. At the same BP, a 4.5-gm force will occlude an artery 1.5 mm in diameter, and a 5.5-gm force will close an artery 2.0 mm in diameter. If the BP is raised to 210 mm Hg, an artery 1 mm in diameter should be occluded by a 6.5-gm force, a 1.5-mm artery by 10.0 gm, and a 2-mm artery by 13.5-gm force (Fig. 9).

These pressure-occlusion values are most likely influenced by the thickness and morphological state of the arterial wall.

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**Fig. 10.** The lever law (Archimedes) states that the product of the force \( f \) applied over a length \( l \) of a lever is equal to the resultant products of Force \( F \) and Length \( L \) on the corresponding opposite end of the lever. \( f \times l = F \times L \). This may be rearranged to give \( f = F \times L / l \). By applying a force to one end of the clip and by knowing the respective lengths, we could calculate the force exerted by the clip. The graph illustrates that as the length from the fulcrum \( l \) decreases, the force exerted by the clip increases dramatically.
Grooves or serrations on a clip's surface are undesirable as these effectively decrease surface area, and there is stress concentration in the vicinity of the sharp edge with a transmission of excessive pressure to the arterial wall directly beneath these ridges. Also, the force exerted on the artery increases exponentially as the distance from the fulcrum decreases (Fig. 10).

A wide, gentle curve to a clip's surface will reduce the shearing force and the strong concentration along the clip's edge. A self-loading, relatively long-jawed clip of soft metal would seem to be inherently desirable. Spring force and surface area should be considered in the designing of temporary clips for microvascular surgery. Indeed, the specific mechanical properties of all the clips used in modern neurosurgery, that is, aneurysm clips, should be determined in a similar manner.

In conclusion, the study suggests that temporary microsurgical clips should be designed, tested experimentally in vivo, and SEM and light microscopy should be used to assess any resulting arterial pathology.

Acknowledgments

The authors express their sincere thanks to the Department of Medical Media of the Veterans Administration Hospital of Pittsburgh, Pennsylvania, to Ms. Aphia Abdou for the drawings, and to Mrs. Gertrude Vohreinger for technical assistance.

References


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