Endothelial lesions after temporary clipping

A comparative study

BERND RICHLING, M.D., GOTTFRIED GRIESMAYR, ALOIS LAMETSCHWANDTNER, PH.D., AND WOLFGANG SCHEIBLBRANDNER, M.D.

Neurosurgical Department, Landesnervenklinik, and Institute of Zoology, University of Salzburg, Salzburg, Austria

The effect of temporary clipping on the arterial endothelium of rats was examined with scanning electron microscopy. The opening pressure of four Heifetz clips was modified by changing the springs. Four different clip forces (20, 35, 45, and 65 gm), four periods of clipping (10, 30, 60, and 180 minutes), and three vessel diameters (smaller than 1 mm, 1.1 to 1.3 mm, and 1.4 to 2 mm) were compared. Different grades of endothelial damage were observed. On gross examination the damage involved a detachment of endothelium and the adherence of platelets to the subendothelial tissue. The duration of clipping seemed to be of more importance than the clip force, whereas the vessel diameter had no recognizable influence.

KEY WORDS: vascular clip, endothelial lesion, clip force, clipping period, aneurysm clip

Since the first description by Cushing in 1911,10 vascular clips have become a necessary part of modern neurosurgery. Changes in shape and material were made in 1927 by McKenzie,27 and in 1950 by Duane.14 In 1953, Norlén and Olivecrona32 developed a wire clip that could be reopened by applying the clip holder posterior to the axis. The next step was a clip that closes by spring force and can be reopened as required. In 1971, Mayfield and Kees28 improved the design of the Schwartz cross-legged clip, and the resulting Mayfield clip has for some time been the most widely used spring clip. During that period, modifications included rugated blade surfaces, overlapping clip tips,4 and silicone rubber-coated clips.26 In 1969, Heifetz19 designed a clip with an internal non-fatiguing wire spring.

With the introduction of microsurgery,29 and especially techniques for vascular anastomoses in the neurosurgical field,21,25 it became even more necessary to pay attention to the proper position of the clip and the exact occlusion of the vessel, and in particular to the spring force of the clip. The danger of endothelial lesions caused by temporary clipping, with the possible complication of thrombosis, required the development of special soft clips.20,22,23,36

Many authors describe the nature and extent of endothelial lesions at the site of microsurgical anastomoses,3,24,28,31 but very few papers deal with the nature of endothelial lesions caused by temporary clipping.2,18,34 Dujovny, et al.,15 have reported acute and chronic endothelial changes after temporary clipping for 45 minutes with spring clips of different designs. The aim of this paper is a comparison of the effects of various clip forces, vessel diameters, and periods of clipping with different endothelial lesions.

Materials and Methods

For this study we used 51 male and four female Sprague-Dawley rats with a body weight ranging from 115 to 496 gm. The animals were anesthetized by a subcutaneous injection of Nembutal (pentobarbital), 50 mg/kg body weight. The animals were put on a heatable operating table; temperature was regulated with an electrode placed into the rectum. The body temperature of the animal was kept constant at 39°C during the experiment. Then one common carotid
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artery, or one common carotid artery and the abdominal aorta, or one common carotid artery and one or both iliac arteries were exposed for clipping. The diameter of the arteries was measured under the dissecting microscope and classified into three groups:

- **Group I**: smaller than 1 mm
- **Group II**: 1.1 mm to 1.3 mm
- **Group III**: 1.4 mm to 2 mm.

The common carotid artery and the iliac artery of young animals fell into Group I, the common carotid artery of adult rats into Group II, and the abdominal aorta of adult rats into Group III.

To test the effect of different spring forces on the endothelium, it was necessary to design clips with varying defined spring forces, but otherwise with identical properties. For this purpose the steel wire springs were removed from four straight, 8-mm Heifetz clips and replaced by new springs. Methods of measuring the opening force of a clip are by a spring scale, a dynamometer, or electronic scales. We used the method suggested by Sugita (Fig. 1). One branch of the clip was glued to a firm surface. A loop of fine steel wire was put over the lower branch at a distance of 7.5 mm from the axis. A tiny bowl was attached to the steel wire to hold the weights. The weight that was able to open the clip 1 mm at the measuring point was used to define the opening force. Four Heifetz clips with new spiral springs were tested three times each by this method. The following values were obtained: 20 gm (Clip A), 35 gm (Clip B), 45 gm (Clip C), and 65 gm (Clip D), with a mean error of ± 3 gm.

The durations of clipping were: $t_1 = 10$ minutes, $t_2 = 30$ minutes, $t_3 = 60$ minutes, and $t_4 = 180$ minutes. All three parameters were combined, resulting in 48 different experiments (four clips × four clipping periods × three diameters). The time was recorded from the first clipping to the removal of the clip. During the clipping procedure the clipped area was protected against desiccation by a covering of surrounding tissue and wet (0.9% NaCl) gauze.

After the designated clipping period, the clip was removed, the chest of the animal opened, and the heart exposed. Five minutes after removal of the clip, the arterial system was rinsed from the left ventricle with buffer solution (sodium cacodylate, pH 7.4, 0.1 M at room temperature) until the efflux from the opened right atrium became clear (within 2 minutes). The subsequent fixation with buffered 2.5% glutaraldehyde at room temperature perfused by the same route lasted 5 minutes. The perfusion pressures were between 115 and 120 mm Hg. The clipped and control arteries were then excised and fixed for 2 more hours by immersion in buffered glutaraldehyde. After two 60-minute washes in buffer solution, the arterial segments were dehydrated in a graded series of ethanol, in ethanol:Frigene II ratios of 2:1, 1:1, and 1:2, and in Frigene 11, and critical-point dried with Frigene 13. The dried specimens were cut longitudinally with razor blades and mounted on Alu-stubs with the lumen uppermost. The arterial segments were then sputtered with gold and examined with a Stereoscan Mark IIa scanning electron microscope (SEM) at an acceleration voltage of 30 kV. From every specimen, survey micrographs at a magnification of × 120 to × 240, and details of special areas were taken for photographic documentation.

**Results**

In most cases the endothelium of the normal abdominal aorta of control animals revealed a folded surface with distinct cell borders. A smooth surface was scarcely seen (Fig. 2 left). In the normal common carotid artery (Fig. 2 right) and in the iliac artery, endothelial folds were also present.

The endothelial damage that occurred with temporary clipping of the arteries with Heifetz clips is graded as follows:

- **Grade 1**: The clipped area could not be identified under the SEM. No significant differences to control specimens are observed (Fig. 3).
- **Grade 2**: Local damage of the endothelium is present. The lesion is much smaller, however, than the width of the clip and results in longitudinal or transverse stripes (Fig. 4 left). The damage is characterized by detachment of endothelial cells.

and by platelet adhesion on the exposed subendothelial tissue (Fig. 4 right).

Grade 3: The endothelial injury extends over the whole circumference of the artery (Fig. 5 left). The disrupted endothelium is removed incompletely. In detail the image is similar to Grade 2, but the dimension of the injured area corresponds approximately with the width of the clip (Fig. 5 right).

Grade 4: The whole width of the clipped area is denuded of endothelium and is covered with a monolayer of uniformly distributed platelets (Fig. 6). The borderlines of the undamaged endothelium proximal and distal to the clipped region are well defined.

In specimens showing Grade 3 or 4 endothelial damage, the position of the clip was often visible un-
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Fig. 4. **Left:** Low-power photomicrograph of the luminal surface of the iliac artery (Ø smaller than 1 mm) showing Grade 2 endothelial damage. Blood platelets are adherent to the vessel wall at some spots. The artery was clipped for 30 minutes with Clip D, a 65-gm spring force. × 170. **Right:** On the higher power view, the desquamation of the endothelial cells becomes evident. The subendothelial tissue shows adhering platelets. × 840.

Under the dissecting microscope at low magnification as a barrel-shaped dilatation of the vessel. Alterations of the longitudinal folds of the lamina elastica interna were present (Fig. 6). This suggested that the effect of clipping was not limited to the endothelium.

The areas proximal and distal to the clipping site seemed to be quite normal and had the same appearance as those in the control preparations. Furthermore, no morphological differences between the distal and the proximal sides were observed. When the clip was applied for a long time (1 and 3 hours) leukocytes appeared at the clipped area (Fig. 6 lower). Fibrin strands were rarely seen, as the time of blood flow after clip removal was rather short. Comparable data on the endothelial damage are presented in Fig. 7. With Heifetz Clip A with a spring force of 20 gm, the increase of clipping time was directly proportional to the degree of intimal lesion. Differences in vessel

Fig. 5. Grade 3 endothelial damage. **Left:** Abdominal aorta. The endothelium is detached over the whole circumference of the vessel, but fragments are still visible. Arterial clipping for 180 minutes with Clip B, a 35-gm spring force. × 170. **Right:** Common carotid artery. The exposed subendothelial tissue is tightly packed with platelets. Arterial clipping for 30 minutes with Clip D, a 65-gm spring force. × 880.
FIG. 6. Grade 4 endothelial damage to the abdominal aorta. **Upper Left:** The clip site is clearly shown by a barrel-shaped dilatation when clipped for 30 minutes with Clip B, a 35-gm spring force. \( \times 45 \). **Upper Right:** The luminal surface is free of endothelial cells, a monolayer of platelets covers the subendothelial tissue. Arterial clipping for 180 minutes with Clip A, a 20-gm spring force. \( \times 880 \). **Lower Left:** Same sample as upper right, showing the invasion of leukocytes. \( \times 175 \). **Lower Right:** High-power view of lower left. Platelets with long processes as well as numerous round leukocytes are attached to subendothelial tissue. \( \times 1750 \).

diameter showed no influence on the degree of lesion. With Heifetz Clip B (35 gm) the influence of clipping time was marked, and vessels of different diameter had varying reactions. The influence of time was less important with Heifetz Clip C (45 gm), and even a short period of clipping led to visible damage. When using the Heifetz Clip D (65 gm), severe endothelial damage was already seen at short clipping times (10 minutes). At a 180-minute clipping period, vessels of all diameters showed the most severe intimal lesions.

The following conclusions can be drawn (Fig. 7): 1) The diameter of the clipped artery cannot be correlated with the grade of the intimal lesion. When a constant spring force was used, different diameters showed totally variable relations to the intimal damage. 2) The spring force of the clip used is important, especially at short clipping times. Here a clear relationship between spring force and endothelial lesion can be seen. 3) The most important factor is the clipping time. This becomes evident with weak spring forces. At a clipping time of 180 minutes, even a small spring force will produce a severe endothelial lesion. With one exception, Grade 4 damage only was always found at 180-minute clipping times.
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Discussion

The endothelial damage seen in our preparations can cause two subsequent events: the short-term formation of a mural thrombus or, in the course of the healing process, a thickening of the intima by massive proliferation of smooth-muscle cells. Several authors report the formation of a thrombus after experimental endothelial injury. It could be demonstrated that thrombus formation reaches its maximum 10 to 20 minutes after injury, whereas no further adhesion of platelets takes place after 30 minutes. After that, leukocytes appear and start to remove the thrombus. A tight monolayer of platelets takes the place of the endothelium.

In agreement with previous authors, we found that, after a clipping time of 3 hours, numerous leukocytes were present at the clipping site (Fig. 6 lower). Therefore, the danger of a thrombotic arterial occlusion has decreased after approximately 30 minutes. The importance of the long-term thickening of the intima for later vascular disease cannot be discussed here.

The longitudinal folds of the endothelial surface are folds of the internal elastic membrane. Under normal preparation procedures they are always present. When tissue is perfused with a fixative under pressure over a long period of time, the endothelial surface becomes smooth and can be studied in some detail. In our experiments a perfusion pressure of 115 to 120 mm Hg was applied for 5 minutes, so a smooth endothelial surface could be obtained only rarely (Fig. 2). The folded endothelial surface was seen predominantly in our control animals as well as our experimental animals. The barrel-shaped dilatation frequently observed at the clipping site (Fig. 6 upper left) led us to the conclusion that the elastic fibers or the media of the vessel had also been injured.

Despite the large total number of histological preparations we examined, we were able to make only one preparation of every combination possible (clipping time, vessel diameter, and spring force). Therefore the histological degrees of lesion obtained are not valuable statistically. Even so, an examination of the results gives clinical information about the influence of time, vessel diameter, and spring force. The fact that clipping time is of greater importance for the degree of endothelial lesion than spring force is of practical interest. In particular it should be emphasized that even the smallest spring forces used over 180 minutes produced severe endothelial lesions. Physical principles (Laplace's law) were challenged by our results. The vessel diameter, which directly influences the extent of surface pressure throughout the area clipped, showed surprisingly little influence on the degree of intimal lesion. Under a constant spring force, vessels with small diameters showed intimal lesions no more severe than vessels with larger diameters. Other authors have already reported this fact. There might be varying properties of vessel walls, which cannot be identified with our experimental protocol, to account for this finding. The importance of the spring force on the degree of intimal lesion is diminished by the overriding influence of the period of clipping. With a long clipping time (180 minutes) both firm and very gentle clips produce severe intimal lesions. With shorter clipping periods the expected connection between spring force and intimal lesion became apparent.

In vascular microsurgery particular attention should be given to the duration of temporary clipping. The use of medium-gentle spring clips (42 to 64 gm spring force) offered for temporary clipping cannot protect against severe intimal lesions if the time factors are neglected. During microvascular surgery the clipping time cannot be reduced at will; therefore, it would seem advisable that the spring force does not exceed the degree necessary for occlusion of the vessel and a secure position of the clip. The use of a greater variety of gentle temporary clips and special attention to time could lead to an optimum result with regard to the endothelium.

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Address reprint requests to: Bernd Richling, M.D., Abtlg.f. Neurochirurgie, Landesnervenklinik, A 5020, Salzburg, Austria.