Scanning Electron Microscopic Observations on the Luminal Surface of the Rabbit Common Carotid Artery Subjected to Ischemia by Arterial Occlusion

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Abstract:
The scanning electron microscope (SEM) has been employed to study the effects of ischemia on the luminal surface of the common carotid artery. Fifteen adult rabbits were lightly anesthetized and the common carotid arteries surgically exposed. The right carotid artery was occluded with a single Heifetz clip for five minutes (five animals), 15 minutes (five animals), and 30 minutes (five animals). Following removal of the clip, the animals were immediately perfused with glutaraldehyde and the arteries excised and prepared for critical point drying. Four additional rabbits were perfused following the same method with no surgical procedures performed in the neck. Normal aortas were also examined. The nature and frequency of endothelial cell alterations were determined by analysis of ten randomly selected SEM fields. Examination of the endothelial surface of arterial segments distal to the occluding clip revealed the presence of numerous "crater-like" defects as well as outpouchings or "balloons." The numbers of craters and balloons were significantly increased in the ischemic (distal) arterial segment as compared to either proximal or sham-operated control segments (P < 0.001). These endothelial cell alterations were never observed in random micrographs of arterial segments taken from unoperated control animals, but were seen at the ostia of some intercostal arteries of the aorta. It is suggested that these craters and balloons could cause interference with blood flow and the formation of platelet thrombi by their protrusion into the lumen, as well as alteration of the permeability of the arterial intima.

Introduction
Most ultrastructural studies of the effects of ischemia upon the central nervous system have been concerned with alterations in small intracerebral vessels or brain parenchyma. The scanning electron microscope (SEM) permits examination of surfaces at a wide range of magnifications and has been employed in a previous study of the effect of proximal and distal occlusion by removable clips on the endothelial lining of the common carotid arteries of the rhesus monkey. The most striking abnormality was the appearance of crater-like formations on the luminal surface. The present study was undertaken utilizing a single removable clip to block blood flow in the common carotid artery in order to study ischemic changes in endothelium in a situation more nearly resembling that occurring clinically in man. In addition, observations described in this report were made on specimens prepared by critical point drying, a technique generally considered superior to air drying for preserving tissue fine structure.

Methods
Fifteen New Zealand white rabbits (1.0 to 2.9 kg) were anesthetized with sodium pentobarbital (Nembutal®, 35 to 40 mg per kilogram; IV) and both common carotid arteries were surgically exposed. A single Heifetz clip was used to occlude the right common carotid artery for five minutes (five animals), 15 minutes (five animals), and 30 minutes (five animals). The clips were then removed and the arteries perfused with 1.2% glutaraldehyde in 0.075 M Sorensen's phosphate buffer (pH 7.4; 320 mOsm; room temperature) by
way of a cannula inserted through the left ventricle into the ascending aorta. The left carotid arteries of these animals were used as control specimens, and segments of the thoracic aorta containing branching points were also examined. In addition, four control animals were perfused following the same method with no surgical procedures having been performed in the neck.

Specimens were removed and immersed in 2.5% glutaraldehyde in 0.1 M phosphate buffer for two hours and stored overnight in 0.15 M phosphate buffer (pH 7.4; 4°C). After postfixation in 1% osmium tetroxide for 45 minutes, tissues were dehydrated in graded solutions of ethanol, placed in increasing concentrations of iso-amyl acetate and dried in a “Polaron” critical point drying apparatus using liquid CO2 as the immersion medium. The specimens were then mounted on stubs and coated with gold palladium, and the luminal surfaces examined in a Cambridge “Stereoscan” scanning electron microscope. A series of ten random microscopic fields was analyzed for the nature and frequency of endothelial cell alterations. These fields were selected at 100X magnification, which is below that necessary to permit detection of endothelial abnormalities. Magnification was then increased to 1,000X for examination and counting of endothelial lesions. This analysis was performed on areas more than 5 mm proximal to the clip and 5 mm distal to the clip on the operated side, and on corresponding areas of the contralateral sham-operated control artery as well as on unoperated control specimens. P values were determined by an analysis of variance procedure.

The possibility of arterial blood reaching the common carotid artery distal to the occluding clip through collateral supply channels was tested in two additional rabbits. The distribution of 65 cc of 30% Pelikan Special Ink Black in saline was observed following its infusion into the inferior vena cava shortly after clipping of the right common carotid artery.

Results

SEM examination of the luminal surfaces of control specimens of common carotid arteries reveals considerable variation in appearance of the endothelium. The endothelial surface is convoluted and irregular in some areas while relatively flat elsewhere except for

![Figure 1](http://stroke.ahajournals.org/)

Normal-appearing luminal surface of a 15-minute sham-operated common carotid artery showing variability of surface topography. C: convolutions resulting from contraction of the internal elastic lamina; E: erythrocyte.
SEM OF THE ISCHEMIC COMMON CAROTID ARTERY

Ovoid protrusions which represent that part of the cytoplasm overlying the nucleus (figs. 1 and 2). The endothelial cells appear fusiform in shape with their long axes parallel to the direction of blood flow. In some specimens the apparent boundaries of individual endothelial cells can be partially visualized (fig. 2), as described by Garsch and Christensen with silver nitrate staining. "Bridge-like structures" such as those described by Shimamoto et al. and Sunaga et al. were sometimes observed in tissues from control animals. They were also present in the area which had been compressed by the clip (fig. 3). These structures are probably related to contraction of the internal elastic lamina and may represent a distortion of the nucleus of the endothelial cell. A rather consistent feature of normal endothelial cells dried by the critical point technique is surface striations oriented perpendicular to the longitudinal axis of the cell (figs. 2 and 3).

Endothelial cells subjected to ischemia (distal to the site of the occluding clip) show two very striking alterations. The first is the presence of numerous "crater-like" defects or holes in the endothelial surface (figs. 4 and 5). Such craters vary considerably in size, shape and distribution. They often appear somewhat broader at their base and usually protrude into the lumen. Occasionally, unidentified particulate material may be seen in the interior of the crater. While craters are frequently observed in single or contiguous endothelial cells, other regions of the luminal surface, also distal to the clip, appear relatively normal. In addition numerous outpouchings or "balloons" protrude into the lumen from the endothelial cell surface in the distal arterial segments (fig. 6a). As with the craters, balloons are present in some areas of the ischemic specimens while adjoining areas appear normal. In most cases the morphological appearance of balloons is that of hemispheric protrusions from the endothelial cell surface. Occasionally, however, the surface membrane of such an

![Image](http://stroke.ahajournals.org/)

**FIGURE 2**

Normal-appearing luminal surface of a 30-minute sham-operated common carotid artery. The long axis of the endothelial cell is oriented in the same direction as part of the artery. N: that portion of the cell overlying the nucleus; CS: cytoplasmic striations. White arrows delineate apparent outline of individual endothelial cell.
Endothelial surface of a common carotid artery subjected to 30 minutes’ clipping (clamp site). B: "bridge-like" structures; G: granulocyte.

Outpouching may appear collapsed or ruptured (figs. 6b and 6c).

The number of craters and balloons was significantly increased in the ischemic arterial segments as compared either to proximal segments or to specimens from the sham-operated control artery (P < 0.001) (table 1). While fewer craters and balloons were seen in the sham-operated controls than in the segment proximal to the clip, this was not statistically significant (P > 0.20). These endothelial cell alterations were never observed by random field analysis of the arterial segments taken from unoperated control animals.

Craters and balloons were observed in endothelium which had been rendered ischemic for as little as five minutes (table 1). No increase in frequency of their occurrence was apparent after 15 minutes of arterial occlusion. Craters and balloons were more numerous following 30 minutes’ clipping; however, the overall effect of increasing periods of ischemia achieved only borderline statistical significance (P = 0.12).

SEM examination of segments of the thoracic aorta revealed similar alterations (craters and balloons) at some branching sites of these vessels (fig. 7). Preliminary observations suggest that these abnormalities are concentrated at branch points with little or no involvement of other areas of the luminal surface. However, more extensive studies will be necessary to confirm this impression and to determine whether there is a consistent relationship to the direction of blood flow or geometry of the site of branching.

Discussion

The scanning electron microscopic characteristics of the endothelial surface of the common carotid artery of the rabbit were found to parallel closely those observed previously in a study of the monkey common carotid artery.4,5 However, cross-striations or bands in the endothelial cell surface were not a consistent finding in the air-dried specimens utilized in that study. Whether any relationship exists between such cross-striations and the poorly understood contractile
mechanism of endothelial cells remains uncertain. The critical point drying technique is generally considered superior to air drying in decreasing cell distortion and disruption, caused by the effects of surface tension in the latter procedure.  

While the occurrence of craters in endothelial cells from arterial segments subjected to ischemia has been reported, the balloon-like structures illustrated in this study have not been described previously. The great variation in frequency and distribution of these abnormalities within ischemic arterial segments of the same and of different animals is puzzling. One possible explanation for differences among animals may be a variation in collateral blood supply and hence in severity of ischemia. The existence of collateral flow in these preparations was easily demonstrated by infusion of carbon black solution into the inferior vena cava following occlusion of the right common carotid artery. The black tracer material was almost immediately visible as a cohesive column entering the distal region of the vessel from above, most likely demonstrating collateral flow through the circulus arteriosus.

A relationship between the formation of craters and balloons has not been proved; however, it seems likely that craters represent ruptured balloons. Some suggestion of this is shown in figure 6; however, verification of this as well as understanding of the mechanism involved will require correlated scanning and transmission electron microscopic studies.

Significantly greater numbers of craters and balloons were found in the endothelial surfaces of the ischemic segment of the arteries (distal to the clip) than in either the proximal region or the sham-operated control specimens (P < 0.001) (table 1). Since these alterations were never observed in random micrographs of unoperated control arteries, it is reasonable to assume that the small number of craters and balloons present in the sham-operated segments may be due to surgical trauma. In the proximal
FIGURE 5

Higher magnification of crater (arrow). T: possible thrombocyte.
F10UU 60

Endothelial surface of a common carotid artery subjected to ischemia for five minutes (distal segment). There is a crater in the upper portion of the field (arrow). Bal: balloon.

FIGURE 6a

Endothelial surface of a common carotid artery subjected to ischemia for 15 minutes (distal segment). Note the margin of the crater suggesting ruptured surface membrane of a balloon (arrow).

FIGURE 6b

Endothelial surface of a common carotid artery subjected to ischemia for 30 minutes (distal segments). Showing crater formation, possibly following rupture of the balloon (arrow).

FIGURE 6c
Luminal surface of a “normal” thoracic aorta at the point of origin of an intercostal artery. The direction of blood flow is indicated by the long arrow. The area indicated by the short arrow is depicted in figure 7b.

Higher magnification of the area indicated by the short arrow in figure 7a. Note the numerous craters (arrows). The enclosed area is depicted in figure 7c.

Higher magnification of the enclosed area in figure 7b.
SEM OF THE ISCHEMIC COMMON CAROTID ARTERY

TABLE 1
Comparison of the Total Number of Craters and Balloons in Distal, Proximal, and Control Segments of Animals Subjected to Ischemia for 5, 15, and 30 Minutes

<table>
<thead>
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<th>Segment</th>
<th>5 minutes</th>
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<th>30 minutes</th>
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<td>B</td>
<td>C</td>
</tr>
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</tr>
<tr>
<td>Average</td>
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</tr>
<tr>
<td>Average</td>
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<td>1.4</td>
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<th>30 minutes</th>
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<tr>
<td>Average</td>
<td>3.2</td>
<td>0.8</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*Craters.
+Balloons.
‡Average number of craters for the five animals.
Each number represents the total number of craters or balloons for ten random fields. (See text for statistical analysis of data.)

segments there may also be rheological stresses resulting from the sudden vascular occlusion.

It has been hypothesized that crater formation is a nonspecific reaction of endothelial cells to injury.4,5 This is supported by the observation that, in addition to ischemia and surgical trauma, similar endothelial cell alterations have been reported in rabbits which were maintained chronically on a high cholesterol diet.14 The possible relationship between endothelial cell injury, blood flow, and atherogenesis and thrombogenesis is a subject of great interest and importance. If alterations such as those described in this report occur in vivo, it is conceivable that the permeability of the arterial intima could be significantly altered at these sites. Such craters and balloons might also contribute to the formation of platelet thrombi by their protrusion into the lumen. Furthermore, if such craters and balloons are present in microcirculatory beds, such as the cerebral capillary network, they would appear to be of sufficient size to cause partial or complete obstruction of the vessel, interference with blood flow, and subsequent ischemia of the parenchyma.

It is of interest that similar alterations were observed at some arterial branching sites of the thoracic aorta, particularly in what Flaherty et al.15 term the "flow-divider" area of the branch point. Since branching sites are known to be areas of predilection for development of atherosclerosis,15,16 one might speculate that the process of atherogenesis may be facilitated by continued damage (such as ischemia, trauma, hyperlipemia or turbulence) to endothelial cells in areas of the blood vessel already rendered "abnormal" by intimal cushion formation.17,18 If such speculations are true, further SEM studies on the nature of endothelial cell alterations, particularly at branch points, may provide a basis for dietary or pharmacological intervention in the prevention of thrombogenesis and atherosclerosis.

Acknowledgments
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References


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