Ultrastructural alterations in blood vessels of the white matter after experimental spinal cord trauma

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The ultrastructure of the microvasculature of the white matter of the spinal cord was studied after experimentally induced trauma in the cat. Immediately after the induction of trauma, disruption of endothelial cell junctions, increased pinocytotic activity in endothelial cells, and perivascular edema were seen in the blood vessels at the site of injury, but not in those of adjacent segments. However, within 2 hours of injury, the blood vessels of the white matter of the rostral and caudal segments also showed evidence of increased endothelial cell pinocytotic activity and perivascular edema, but disruption of endothelial junctions was not seen. It is therefore concluded that vasogenic edema occurs in the white matter at the site of injury soon after trauma and is due both to leakage from vessels with damaged endothelial cell junctions and also to increased transvesicular transport. By contrast, vasogenic edema develops only after a lapse of time in segments rostral and caudal to the site of injury, and probably results from increased transvesicular transport. A possible role for neurogenic chemical mediators in the genesis of the perivascular edema is discussed.

KEY WORDS: microvasculature • spinal cord trauma • perivascular edema • white matter • ultrastructural study

ALTERED blood flow and local edema following experimental spinal cord injury are thought to have profound effects on the morphological and functional state of the spinal cord.5,14 Development of ischemia 2 hours after injury at a site distal to the impact has been shown as a delayed pathological event.16,17 This delay would make available sufficient time for invaluable therapeutic intervention.

In a previous study,13 it has been established that, following spinal cord trauma, edema develops in the gray matter at the site of injury and subsequent extravasation occurs into the white matter, presumably by bulk flow. Further studies18-20 have shown that over a period of time the edema in the white matter spreads to segments proximal and distal to the site of injury.

Ultrastructural changes in the microvasculature of the gray matter at the site of spinal cord trauma have been described in animals injected with horseradish peroxidase after induction of trauma.2,3,7-9 These changes consist of perivascular edema accompanied by increased pinocytotic activity2,3,8,9 and endothelial cell separation.7 However, no information currently exists as to whether morphological changes in the blood vessels of the white matter occur at the site of injury and whether similar changes develop in proximal and distal segments with time. The occurrence of such changes could explain the delayed formation of edema at a distance from the site of injury. Much of the progressive damage known to occur in spinal cord injury, resulting in severe and permanent neurological deficits, have been attributed to the development of edema.

The present investigation was undertaken to examine whether alterations in the microvasculature of the white matter of the spinal cord develop after experimentally induced trauma.

Materials and Methods

Adult cats, each weighing 3 to 4 kg, were anesthetized with sodium pentobarbital. A laminectomy was performed exposing vertebral segments T5-9. Spinal cord trauma was induced with a standard 500 gm-cm contusion at the T-7 level. Some animals were sacrificed immediately after impact, while others were kept alive for a period of 2 hours. Untraumatized spinal cord was used for control studies. All animals were perfused intracardially with a mixture of 4% paraformaldehyde and 0.2% glutaraldehyde in 0.12 M Millonig's phosphate buffer, pH 7.2. One hour after perfusion, the spinal cord was removed, and cross sections, 1 to 2 mm thick, were taken at segmental levels T-7 (site of trauma), T-6 (proximal to the site of trauma), and T-8 (distal to the site of trauma). From each section, representative areas from the dorsal, lateral, and ventral
white columns were dissected out under the dissecting microscope. The dissected tissue was left in the same fixative in the cold for 1 hour and then transferred into phosphate buffer. The tissue was osmicated using 2% osmium tetroxide, dehydrated, infiltrated, and embedded in Epon. Sections were cut 0.5 μm thick on an ultramicrotome, stained with toluidine blue, and carefully examined for blood vessels. Serial thin sections were then taken, stained with uranyl acetate and lead citrate, and examined under a Phillips 201 electron microscope.

Results

Control Animals

The normal vascular morphology in the white matter of the spinal cord from an untraumatized animal is presented in Fig. 1. A segment of blood vessel with endothelial cells lining the lumen is seen. There is a distinct paucity of pinocytotic vesicles in the endothelial cells. The perivascular space contains a few collagen fibers and pericytes. The space is bounded by the inner and outer layers of basement membrane indicated in

FIG. 1. Electron micrograph of part of a capillary in the white matter of nontraumatized spinal cord. Endothelial cells (E) line the lumen (L). The perivascular space is flanked by an inner lining of basement membrane (arrowheads) adjacent to the endothelial cell and an outer lining of basement membrane (crossed arrows). The perivascular space contains some collagen fibers (asterisks). The outer basement membrane is surrounded by glial processes (GP). P = pericyte. × 40,500.

FIG. 2. Electron micrographs of a capillary taken from the site of trauma immediately after injury. Left: Endothelial cell (E) morphology is intact. There is separation of endothelial junction (arrow). The perivascular space (PVS) appears damaged. There is a swollen pericyte (P). The asterisk indicates an axon profile in the perivascular space. × 9720. Right: High-power view to show the ballooned out endothelial junction (arrow) and the axon profile (asterisk) in the perivascular space (PVS), seen left. × 32,400.
Blood vessel alterations in cord trauma

FIG. 3. Electron micrograph of a capillary seen at the site of trauma immediately after injury. The apparent increase in the perivascular space (PVS) is a result of the collapsed lumen (L). The endothelial cell (E) and its junctions are normal. A swollen pericyte (P) is seen in the field. Arrows indicate intact basement membrane. x 12,150.

Fig. 1 by arrows. Many glial processes abut against the outer layer of the basement membrane.

Changes in Vessels Immediately After Trauma

At the site of spinal cord injury (T-7), the microvasculature (capillaries, pericytic venules, and arterioles) in the white matter showed the following morphological appearance. 1) Many vessels appeared normal, with preservation of the basement membrane. The endothelial subcellular morphology was also preserved, with intact endothelial tight junctions. 2) The perivascular space in some of the capillaries showed evidence of disruption (Fig. 2 left). Figure 2 also shows an axon profile close to the capillary wall. 3) Some vessels were distorted, with their lumens collapsed. In these cases the vessel wall was clearly separated from the outer layer of basement membrane (Fig. 3), but with no morphological evidence of perivascular edema. The subcellular morphology of the endothelial cells in these vessels was preserved; however, some of the pericytes appeared swollen (Fig. 3). In some severely distorted vessels, the endothelial cell junctions were also grossly disrupted. 4) There were also many vessels that showed an increase in the number of pinocytotic vesicles in the endothelial cells. The pinocytotic pits were seen along both the luminal and abluminal margins. There was no perivascular edema in these vessels (Fig. 4), yet other

FIG. 4. Electron micrographs of an arteriole at the site of injury taken soon after trauma. Left: The endothelial morphology is preserved with intact junctions (large arrows). There is increased pinocytotic activity. Perivascular space (PVS) and basement membrane are preserved. Small arrows indicate pericytes. L = lumen. x 6075. Right: Higher-power view of the enclosed area seen left. It shows preserved endothelial junction (arrow). The small arrows indicate pinocytotic vesicles at both the luminal and abluminal surfaces. X 20,250.
vessels in the white matter showed increased pinocytotic vesicles in the endothelial cells. In addition there was partial separation of the endothelial junction that formed a distinct space (Fig. 5). The perivascular space in these vessels was widened and was filled with a flocculent homogeneous material probably representing protein exudate characteristic of perivascular vasogenic edema. Some cellular debris was also present in the perivascular space (Fig. 5 left). The basement membrane was intact. Rostral and caudal to the site of injury at segmental levels T-6 and T-8 the vessels were normal.

**Vascular Changes 2 Hours After Impact**

At the site of trauma (T-7), the alterations in the vessel walls 2 hours after impact were identical to those observed immediately following trauma (Figs. 2 to 5). Rostral (T-6) and caudal (T-8) to the site of injury in the dorsal, lateral, and ventral white columns, increased pinocytotic vesicles were observed in the endothelial cells of the vessels (Fig. 6). Perivascular edema and some cellular debris were seen in the perivascular space (Fig. 6). The endothelial junctions in these vessels appeared to be intact. There were many vessels at these segmental levels with normal vascular morphology, however.

**Discussion**

In a previous study, in which spinal cord trauma was induced in cats with methods identical to the ones used in the present study, Stewart and Wagner18 showed that extravasation of fluid occurred in the white matter, not only at the site that was traumatized but also in proximal and distal segments. In that study, the presence of edema in the white matter was demonstrated using fluorescence microscopy, after fluorescein-labeled macromolecules had been intravenously injected into animals 10 minutes prior to the induction of spinal cord injury. The authors explained the occurrence of the edema in rostral and caudal segments as being due to the longitudinal bulk movement of fluid that had extravasated from injured blood vessels at the site of trauma. They further explained the distribution of the fluorescein label, which was more intense in certain locations than in others, as being the result of bulk fluid movement being constrained by the anatomical arrangements of fiber bundles in the white matter. The question as to whether the edema that develops in segments rostral and caudal to the site of injury could

![Fig. 5. Electron micrographs of a capillary, from the site of injury, taken immediately after trauma. Left: Endothelial cell (E) morphology is preserved. One of the two endothelial junctions show separation (arrow). The perivascular space (PVS) is filled with a flocculent material representing edema. CD = cellular debris in the PVS. The basement membrane (small arrows) is intact. × 11,540. Right: High-power view to show the endothelial cell junction separation (arrow) seen left. L = lumen. × 40,500.](image)
be vasogenic in origin had not previously been examined.

In the present investigation, morphological evidence suggesting perivascular edema was noted in the white matter immediately after the cord was traumatized, but this perivascular edema was confined only to the segment that was subjected to trauma. However, after only 2 hours, perivascular edema was observed in proximal and distal segments also.

The results of the present study also throw light on the pathogenesis of the perivascular edema. At the site of trauma, the endothelial cells lining several of the microvessels of the white matter showed one or both of two characteristic morphological changes: disruption of endothelial cell junctions and increased pinocytotic activity. Every vessel that showed morphological evidence of perivascular edema also showed evidence of either disruption of the endothelial cell junctions or increased pinocytotic activity. Increased pinocytotic activity in the microvasculature of the brain has previously been shown to be indicative of increased vesicular transport of fluid out of the vessels.\(^2\)\(^7\)\(^\text{10}\)\(^\text{15}\) It would thus appear that extravasation of fluid occurs at the site of spinal cord injury as a result of damage to endothelial junctions and also due to increased transvesicular transport.

It is interesting to note that, when segments of the cord rostral and caudal to the site of trauma were examined immediately after the induction of injury, not only was there no evidence of perivascular edema, but no morphological changes were observed in the microvasculature of the white matter. However, when these rostral and caudal segments were studied 2 hours after the cord was traumatized, at a time when perivascular edema was present in these segments, then marked evidence of increased pinocytotic activity in endothelial cells lining the microvessels of the white matter was seen. No evidence of disrupted junctions was noted in rostral and caudal segments, even in the presence of perivascular edema. Again, every vessel that showed evidence of exudation in the perivascular space also showed increased pinocytotic activity. Thus, it is apparent that in segments adjacent to the site of cord injury vasogenic edema does develop as rapidly as 2 hours after injury. Increased vesicular transport appears to be the pathogenic mechanism responsible for the genesis of edema.

The reasons for the increase in vesicular transport of fluid into the perivascular space, after a lapse of time, in segments proximal and distal to the site of cord trauma remain speculative. Neurally mediated chemical substances may well be responsible for the development of this vasogenic edema. Both substance P and serotonin have been shown to increase vascular permeability in neural tissue.\(^5\)\(^\text{21}\) Furthermore, in other areas of the central nervous system, dendrites with substance P and serotonin-like immunoreactivity have been described in very close proximity with blood vessels, impinging directly onto capillary basement membrane.\(^1\)\(^\text{11}\) In addition, previous studies have shown that, after spinal cord transection, serotonin accumulates above the level of the lesion\(^\text{14}\) and substance P below it.\(^\text{12}\) It is possible, therefore, that peptidergic and/or amnergic chemical mediators may play a role in the development of edema that is seen to occur in the white matter of the spinal cord at areas proximal and distal to the site of trauma. Further investigation into this area is warranted, as the ability to control the development of edema in the cord may well minimize the extent of irreversible clinical damage that occurs following cord trauma.

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References


![Fig. 6. Electron micrograph showing part of a capillary wall taken proximal to the site of injury 2 hours after induction of trauma. Endothelial cell morphology is intact, but there is increased pinocytotic activity (arrows). The perivascular space (PVS) is filled with a homogeneous material representing edema. The basement membrane is preserved (large arrows). CD = cellular debris in the PVS; L = lumen. × 21,375.](image-url)


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