Ultrastructural blood-brain barrier alterations and edema formation in acute spinal cord trauma

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Endothelial changes leading to edema formation are examined in the primate spinal cord (Macaca mulatta) following a lesion created by a 20-gm weight falling 15 cm onto the exposed dura. Intravascular perfusion of a paraformaldehyde-glutaraldehyde solution followed by carbon black provides adequate fixation of vascular structures and glial elements. Myelin is poorly preserved. Ultrastructural alterations of the blood-brain barrier consist of loss of integrity of the endothelial tight junctions. Edema caused by vascular disruption and parenchymatous extravasation of intravascular contents is observed along with glial swelling. Interglial gap junctions persist in areas of marked cellular separation and do not impede the migration of edema fluid.

Key Words: blood-brain barrier • spinal cord injury • edema • central nervous system injury • endothelial tight junctions

HEMORRHAGIC necrosis following spinal cord injury results from vascular disruption and extravasation of erythrocytes which is more severe in gray than white matter. This study was undertaken to further investigate endothelial changes that result in edema formation, the nature of the edema present, and changes in the blood-brain barrier system during the acute phase of the progressive destructive process.

Materials and Methods

Adult male rhesus monkeys were anesthetized with phencyclidine HCl (Sernylan) and maintained on a mixture of N₂O and O₂ 4:1 by means of a Harvard respirator.* End-expiratory CO₂ was monitored† and maintained within a physiological range. A thoracic laminectomy of T4-6 was performed, and the dura exposed. The spinal cord with intact dura was subjected to an injury created by a 20-gm weight falling 15 cm onto an impactor as described by Albin, et al.¹ This by convention is designated a 300 gm-cm impact. Animals were sacrificed at 1½, 5, 30,
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and 90 minutes, and 6 hours following injury by gravity perfusion through the left ventricle after a median sternotomy and clamping of the distal aorta at the bifurcation. A pressure of 120 cm was used, and blood and excess perfusate were removed by opening the right atrial cavity. The initial perfusion was performed with Karnovsky's solution$^{13}$ diluted 1:3 with 0.1 M cacodylate buffer with the pH adjusted to 7.4, followed by 750 cc of cold concentrated fixative. The spinal cord was then removed, sliced into 1- to 2-mm sections for additional immersion fixation in concentrated fixative for 5 hours. Sections of cord were diced into 1-mm cubes and placed in 0.1 M cacodylate buffer for 12 hours, rinsed briefly in water, post-fixed in 1.3% osmium in S-collidine for 1 hour, followed by uranyl acetate staining en bloc.$^4$ Tissue was then dehydrated through graded alcohol and embedded in araldite. Thick and thin sections were cut by glass and diamond knives respectively on a Sorvall MT-2 ultramicrotome.$^*$ Selected thin sections were post-stained with lead citrate$^t$ and examined with a Philips 301 electron microscope.$^t$ Tissues adjacent to those used for electron microscopy were embedded in paraffin for hematoxylin and eosin staining and light microscopy.

In the traumatized animals the initial perfusion fixation was followed immediately by perfusion through a Y connector with a carbon black dispersion$^1$ containing 10% carbon black (particle diameter 200 to 500 Å), 4.3% fishglue, and 0.9% phenol. Control tissue was obtained from two uninjured animals that received no carbon perfusion and from the lower thoracic cord of injured animals.

**Results**

**Control Tissue**

Control tissue had the normal appearance of central nervous system ultrastructure. Cell membranes were generally preserved and intracytoplasmic organelles were well visualized. Myelin sheaths consistently showed dissolution of the lamellar structure. Endothelial tight junctions with the typical pentalaminar appositions and adjacent increased cytoplasmic density were present in all vessels examined throughout both gray and white matter (Fig. 1). A uniformly dense basement membrane was present surrounding vessels of gray matter with some of the larger vessels showing a collagen-containing perivascular space. A mixed investment of fibrous and watery astrocytes and neuronal processes surrounded gray matter vasculature. This was in contrast to vessels found in white matter which tended generally to be greater than capillary size and usually had an appreciable collagen-containing perivascular space. White matter perivascular glia was predominantly fibrous in nature (Fig. 2). The extracellular space shown by this type of fixation was small (200 to 400 Å between processes); it probably does not represent the true extracellular space but most likely results from influx of fluid into the cells during the fixation process.

**Traumatized Tissue**

In traumatized tissue, alterations in the endothelial tight junctions in gray matter were seen as early as 1½ minutes following injury. Separation of the pentalaminar structure into two distinct bilayered components could be seen at sites of persistent cytoplasmic density, where presumably the membranes had once been in close apposition (Fig. 3). This change occurred in vessels of all diameters ranging from capillary size with no investing smooth

*Fig. 1. Photomicrograph of a normal endothelial junction with fusion of external membrane leaflets to form pentalaminar structures at several points along its course. This demonstrates an anatomic substrate of the blood-brain barrier system. L = lumen, P = pericyte, BL = basal lamina. H & E, × 60,000.*
Fig. 2. Control vessel in white matter showing a prominent collagen (C) containing perivascular space, endothelial junction (arrow), and fibrous glia. H & E, × 4600.

Fig. 3. Endothelial junctions with clear separation of unit membrane structure at sites where cytoplasmic density remains (arrows) shows early changes in the blood-brain barrier. Changes of this type occur within 1 1/2 minutes following injury. H & E, × 55,000.

Fig. 4. Total separation of endothelial junctions with carbon particles present in vascular lumen and extending to basal lamina (BL). H & E, × 35,500.

Edema Patterns

Enlargement of the extracellular space within and adjacent to the hemorrhagic region is consistent with vasogenic edema described by Klatzo. A flocculent material resembling serum protein was present in the extracellular space with varying density from area to area (Figs. 5, 6, and 7). Some regions showed massive enlargement of the extracellular compartment with high concentrations of the proteinaceous material. Enlargement of the cellular processes also occurred close to the lesion where vasogenic edema formation occurred secondary to vascular leakage (Fig. 6). Glial swelling in gray matter is shown in Fig. 8 in an area near the central hemorrhage with only slight enlargement of the extracellular space.

Gap Junctions

Interglial gap junctions occurred in both gray and white matter. The configuration was that of a smoothly curving site of cellular apposition. High resolution microscopy confirmed a septilaminar configuration with separation of the median dense lamina. After this experimental injury these junctions persisted in areas of cellular separation and edema formation, and the seven-layered appearance was retained (Fig. 7).

Discussion

An early concept of a blood-brain barrier system was based on the observation that ex-
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Fig. 5. Early edematous changes in central gray matter near normal-appearing endothelium. Enlarged space occurs between processes in neuropile. Normally with this type of fixation the neuropile contains a small extracellular space with 200 to 400 Å spacing between processes. H & E, × 17,000.

Fig. 6. White matter vessel adjacent to area of hemorrhagic necrosis. A dense flocculent material appears in the enlarged extracellular space (ECS) and perivascular space. Swollen glia (G) with dissolution of fibrils is evident. Perivascular structures include smooth muscle (SM), pericyte (P), and collagen (C). H & E, × 4550.

Fig. 7. Photomicrograph of edematous area in central gray matter. Gap junctions are maintained (arrows) and retain a septilaminar structure (inset, × 75,000). The extracellular space (ECS) is enlarged and contains proteinaceous material. H & E, × 7500.

Exogenous staining agents penetrated the substance of cerebral tissues poorly or not at all while they penetrated other tissues readily. Development of this concept involved identification of an anatomic substrate which rendered the brain parenchyma impervious to foreign tracers. One component of a barrier system has been attributed to the cerebrovascular endothelium. Structural characteristics of the brain cerebrovascular system include imbrication at the point of endothelial junctions. Endothelial tight junctions representing end positions or fusions of the external leaflets of the cell membranes occupy a large portion of the intercellular cleft. There also appears to be a paucity of transendothelial passage of tracer substance by vesicular transport. Presumably proteins and large molecules are impeded in a similar manner. Certain areas of the brain, including the area postrema, neurohypophysis, pineal body, and choroid plexus, allow passage of exogenous tracers into the parenchyma. These regions have an enlarged perivascular space with surrounding connective tissue elements. Evidence that the barrier to protein and molecular structures is due to endothelium rather than the pericapillary space has been presented by Bodenheimer and Brightman. The tight junctions which organize cells into limiting sheets or protein barriers are found only in the endothelium of leptomeningeal and parenchymal vessels and the epithelium of the choroid plexus. The ependyma overlying the area postrema and median eminence also has been found to be
FIG. 8. Photomicrograph shows central gray matter with swollen glial processes (G). Edema of this type presumably results from altered membrane permeability and fluid influx into the cell. H & E, × 7500.

connected by pentalaminar junctional complexes.4,24

Increased vascular permeability resulting from local tissue injury has become an established concept. This leakage is presumably due to direct tissue injury and secondary release of endogenous compounds which contribute to continuing permeability. Histamine and serotonin in striated muscle, for example, have been shown to affect venules. Histamine-produced vascular permeability does not appear to be due to basement membrane changes. Vascular leakage results from partial disconnection of endothelial cells; once these leaks occur, the basement membrane acts as a filter and allows passive diffusion of water but retains serum proteins and tracer particles.20 Brain injury results in escape of fluids into the interstitium; this has been related to permeability changes in the blood-brain barrier system.3,5,9-11,17-19,21,22,26,27

Vasogenic edema, as defined by Klatzo,16 is characterized by increased vascular permeability and an expanded extracellular space containing fluid of high protein content. In cytotoxic edema, the predominant feature is cellular swelling with preservation of the blood-brain barrier system and edema with fluid of low protein content. The intracellular edema is caused by the cells imbibing fluid due to altered membrane permeability.

Impact injury of the primate spinal cord results in edema formation that ultrastructurally shows both vasogenic and cytotoxic criteria. The increase of the extracellular space is due to leakage of intravascular fluid caused by vessel disruption and alteration of the endothelial tight junctions. This could result from two mechanisms. Complete disruption of vessels results in extravasation of blood, which migrates through the parenchyma to expand the extracellular compartment. Also, alteration of the blood-brain barrier system with opening of the endothelial tight junction may provide a site for exit of serum into areas where edema formation occurs. Ultrastructural comparison of the sizes of intra- and extravascular compartments depends on methods of fixation; spaces range from 3% to 5% with conventional methods,12 to as high as 25% with freeze substitution techniques as applied by Van Harreveld, et al.20 Relative differences in fluid compartments are interpreted with the realization that what is seen probably does not actually represent the in vivo spaces. Torack, et al., have examined cortical edema adjacent to a cold-induced lesion and described glial enlargement, concluding that intracellular edema predominated. Lee and Bakay16 have made similar observations concerning gray matter. Their study also included white matter, in which they noted intercellular enlargement in addition to the glial swelling. Protein-rich edema fluid appeared to spread throughout the brain by both intracellular and extracellular routes. Stab wounds of the cerebral cortex cause edema fluid to permeate both white and gray matter extracellular spaces, with the trauma resulting in alteration and leakage of the endothelial junctions at sites remote from the lesion.10

Junctions consisting of seven component layers with a straight or curved, smoothly undulating contour are present between neurons, glia, and ependymal cells. Uranyl acetate staining en bloc enhances the structural definition of junctions of this type which characteristically do not encircle cells.4,24 Lanthanum is capable of outlining the substructure of these areas and demonstrates an array of polygonal subunits of about 90 Å center to center and outlined by a rim of lanthanum approximately 20 Å wide. Functionally these areas are considered to be sites of cellular adhesion, ion transport, or elec-
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trical transmission. It is postulated that the structure of the gap junction allows for inter-
cellular cytoplasmic connection by pores providing for transport of ions and metabolites from cell to cell. Lanthanum permeates spaces formed by the plasma mem-
brane surrounding the pores through which continuity of cytoplasm occurs between two
cells. This rather complicated structure has been described in detail by Pappas.\textsuperscript{28} Suscep-
tibility to proteolytic enzymes and freeze
fracture techniques as demonstrated by
Friend and Gilula\textsuperscript{8} suggests that gap junctions
share a membrane that allows for ionic and
metabolic coupling.

Junctions of this type are identified
between astrocytes of both gray and white
matter of the primate spinal cord after perfu-
sion fixation and uranyl acetate en bloc stain-
ing. The seven-layered structure is apparent,
indicating close apposition but not fusion of
the membrane outer leaflets. After traumatic
injury, these junctions persist in areas of ex-
tensive vasogenic edema and cell separation.
The septilaminar structure and smooth con-
tour are maintained throughout the points of
contact; this is in keeping with one of the
postulated roles of gap junctions as plaques of
cellular adhesion. An extensive study of mem-
branous appositions of the vertebrate brain
by Brightman and Reese\textsuperscript{4} provides evidence
that gap junctions, or maculae occludentes,
interconnecting neural elements, and glia do
not serve as a barrier to proteins.

Summary

Acute blood-brain barrier changes that
follow spinal cord trauma involve loss of in-
tegrity of the endothelial pentalaminal junc-
tions in the central gray matter. This is evi-
dent within 90 seconds following injury and
appears to contribute to edema formation
along with leakage from totally disrupted
vessels. The evidence of cellular swelling and
enlargement of the extracellular space indi-
cates that the spinal cord edema is both
cytotoxic and vasogenic. Interglial gap junc-
tions function as plaques of cellular adhesion
and do not impede the spread of extracellular
fluid.

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