Original article

Folia Neuropathologica

Ultrastructural pathology of endothelial tight junctions in human brain oedema

Orlando J. Castejón

Biological Research Institute "Drs. Orlando Castejón and Haydée Viloria de Castejón", Faculty of Medicine, University of Zulia, Maracaibo, Venezuela

Folia Neuropathol 2012; 50 (2): 118-129

Abstract

Cortical biopsies of patients with the diagnosis of complicated brain trauma, congenital hydrocephalus, brain vascular anomaly, and brain tumour are studied with the electron microscope using cortical biopsies of different cortical brain regions to analyze the alterations of endothelial junctions, and their participation in the pathogenesis of human brain oedema. In moderate oedema, most endothelial tight junctions are structurally closed and intact, while in some cases of severe oedema, the opening of tight endothelial junctions is observed. In very severe brain oedema, a considerable enlargement of interjunctional pockets of extracellular space is also seen suggesting that in highly increased cerebrovascular permeability, the endothelial junctions are open in their entire extent, and that an intercellular or paracellular route through interendothelial clefts for transferring haematogenous oedema fluid from blood to the capillary basement membrane and the brain parenchyma is formed, contributing to the formation of brain oedema. High intensity brain trauma, seizures, osmotic forces, hypoxic conditions, and alteration of tight junctions proteins would explain the opening of endothelial junctions in severe and complicated brain oedema. In congenital hydrocephalus, the capillary wall shows evident signs of blood-brain barrier dysfunction characterized by closed and open interendothelial junctions, increased endothelial vesicular and vacuolar transport, thin and fragmented basement membrane with areas of focal thickening, and discontinuous perivascular astrocytic end-feet. The perivascular space is notably dilated and widely communicated with the enlarged extracellular space in the neuropil, showing the contribution of damaged endothelial junction to the formation of interstitial or hydrocephalic brain oedema. Altered expression of tight junction proteins could cause a blood-brain barrier breakdown following brain injury and hypoxic conditions leading to brain oedema. The results are compared with those found in experimental brain oedema. Some controversial results are also described.

Key words: *endothelial junction, brain oedema, hydrocephalic oedema, brain trauma, brain tumours.*

Introduction

Electron microscopic tracer studies have located the mammalian blood-brain barrier to proteins at the level of tight junctions between endothelial cells of cerebral blood vessels [61]. Earlier electron microscopic studies on brain oedema suggested that the breakdown of the blood-brain barrier was mainly due to enhanced vacuolar and vesicular transport rather than to opening of endothelial junctions [58,69,79], supporting Klatzo's concept [37], who postulates that it is unlikely that

Communicating author:

Dr. Orlando J. Castejón, Instituto de Investigaciones Biológicas, Facultad de Medicina, Universidad del Zulia, Aparatado No. 526, Maracaibo, Venezuela, phone/fax 58-261-7414370, e-mail: ocastejo@cantv.net

the opening of tight junctions may play a significant role in conditions associated with brain oedema. However, subsequently electron microscopic studies have demonstrated that opening of tight junctions may be operative in a variety of neuropathological conditions associated with brain oedema, such as osmotic opening, endothelial contraction induced by histamine-type mediators, and traumatic serotonin release [38]. Goodman *et al.* [28] reported loss of integrity of endothelial junctions after acute spinal cord trauma. Shibata *et al.* [70,71] described open endothelia junctions in cerebral oedema associated with glioblastoma, astrocytoma and meningioma. Alterations of endothelial junctions in the human oedematous cerebral cortex associated with brain trauma, human hydrocephalus and tumours were earlier reported by Castejón [8,11-13] and Glees *et al.* [26]. On the other hand, Kato *et al.* [36] found intact endothelial junctions in rats with brain oedema and encephalopathy due to acute hepatic failure. Nakawaga *et al.* [48] found leaking endothelial junctions for water and ions following acute cerebral ischaemia after occlusion and reperfusion in rats. Sampaolo *et al.* [67] reported that interendothelial clefts of venules and capillaries are the early sites of ischaemic damage. Bullock *et al.* [5] found disrupted tight endothelial junctions following human cerebral contusion. Mossakowsky *et al.* [43] described open endothelial junctions in global cerebral ischaemia in rats due to experimental cardiac arrest. According to Hirano *et al.* [31], the tight junctions of cerebral endothelial cells open under several conditions, such as infusion of hyperosmolar solutions. Urakawa *et al.* [75] reported disruption of tight endothelial junctions in brain capillaries after localized hyperthermia in rats. Baldwin *et al.* [1] demonstrated blood-brain barrier breach after rat cortical contusions. Vaz *et al.* [76,77] reported intact tight endothelial junctions in brain microvessels of patients with traumatic cerebral contusion. Jaeger and Blight [34] found non-overlapping endothelial cell junctions separated by clefts after spinal cord compression injury.

Liebner *et al.* [40] reported the loss of expression of claudin-1in the majority of vessels of human glioblastoma multiforme, which is related with the increase of endothelial permeability. Fischer *et al.* [21] found that 24 h of hypoxia disrupted the continuity of tight junction protein zonula occludens-1 (ZO1), which lines the cytoplasmic face of intact tight junctions. Preston *et al.* [57] described the blood-brain barrier and tissue injury in a model for focal trauma in the rat. Papadopouslo *et al.* [53] and Davies [20] demonstrated open endothelial junctions in high grade astrocytomas, which exhibited loss of occludin expression. Mark and Davis [41] showed alterations in occludin, ZO-1, and ZO-2 in tight junctions induced by hypoxia-reoxygenation. Fischer *et al.* [22] reported that hypoxia increases the paracellular flux across the cell monolayer via the release of endothelial growth factor (VEGF), which in turn leads to the dislocation, decreased expression, and enhanced phosphorylation of ZO-1. Nico *et al.* [50] described open tight junctions in brain capillaries of dystrophin-deficient mdx mouse, an experimental model of Duchenne muscular dystrophy, with disturbance in alpha-actin cytoskeleton, ZO-1, claudin-1, and aquaporin (AQ4) assembly. Gao and Shivers [25] established a correlation between the presence of the blood-brain barrier, tight junctions and expression of zonula occludens protein ZO-1. On the other hand, Hamm *et al.* [30] studying an in vitro model of the blood-brain barrier found that opening of endothelial tight junction was not accompanied by any visible change in the molecular composition of endothelial tight junction proteins, including claudin-3 and 5, occludin, ZO-1 and ZO-2, or β-catenin. Fischer *et al.* [23] demonstrated that hypoxia and VEGF-induced permeability depends on activation of phospholipase C gamma, phosphatidylinositol 3-kinase, and protein kinase G (PKG). Nagy *et al.* [47] described in ultrastructural studies the opening of paracellular avenues in the course of vasogenic oedema in different experimental models. Vorbrodt *et al.* [78] found altered expression of some interendothelial junctions in the brain microvessels of diabetic scrapie-infected mice. Kalini *et al.* [35] concluded that degeneration of noradrenergic fibres from the locus coeruleus causes tight-junction disorganization in the rat brain. Date *et al.* [19] demonstrated a decreased expression of occludin and ZO-1 protein after sustained cerebral ischaemia. Cheng *et al.* [18] reported absence of tight junctions between microvascular endothelial cells in human cerebellar haemangioblastoma. Padden *et al.* [52] found differences in the expression of junctional adhesion molecules-alpha and β-catenin in multiple sclerosis brain tissue. Romanitan *et al.* [63] described overexpression of occludin in Alzheimer's disease and vascular dementia. Rosenberg and Yang [64] demonstrated vasogenic oedema due to tight junction disruption by matrix metalloproteinases (MMP) in cerebral ischaemia. Under ischaemic stroke conditions, decreased blood-brain barrier tight junction integrity results in increased paracellular permeability, directly contributing to cerebral vasogenic oedema, haemor-

rhagic transformation, and increased mortality. Blood-brain barrier disruption, resulting from loss of tight junctions and activation of matrix metalloproteinases (MMPs), is associated with oedema formation in the ischeamic stroke [66].

Cocultured glioblastoma cells and glioma-derived factors (e.g. transforming growth factor β 2) enhanced the paracellular flux of endothelial cell monolayers in conjunction with downregulation of the tight junction proteins. The glioma-derived factors may induce MMPs and downregulate endothelial tight junction protein and, thus, play a key role in glioma-induced impairment of the blood-brain barrier [33].

Research in the last decade has demonstrated that the integral membrane proteins of cerebral endothelial tight junctions are claudin, occludin, and junctional adhesion molecule (JAM). Altered expression of these tight junction proteins could cause a BBB breakdown following brain injury leading to oedema. A significant decrease in JAM-A expression was noted at the lesion site by immunoblotting, supporting the evidence that JAM-A contributes to tight junction integrity [81].

A chemokine, CCL2, induces tight junction disassembly. Disturbance of the tight junction complexes between brain endothelial cells leads to increased paracellular permeability, enabling leukocyte entry into inflamed brain tissue and also contributing to oedema formation. Claudin-5 and occludin, imaged by fluorescent microscopy with simultaneous measurement of transendothelial electrical resistance, become internalized via caveolae and are further processed to early and recycling endosomes but not to late endosomes. The above-mentioned findings suggest that the management of cerebral oedema requires a comprehensive approach in which pharmacologic treatments play a central role. Promising targets include modulators of endothelial cell tight junction proteins, and of aquaporin channel expression within the blood-brain barrier [73].

In experimental conditions, hypoxia-induced oedema formation is mediated by MMP-9-dependent tight junction rearrangement by a mechanism involving vascular endothelial growth factor (VEGF) [2]. More recently, Zhang *et al.* [82] have demonstrated that intravenous injection of anti-HMGB1 monoclonal antibody (mAb) remarkably ameliorated brain infarction induced by middle cerebral artery occlusion in rats. According to these authors, transmission electron microscope observation revealed that the mAb strongly inhibited astrocyte end feet swelling, the end feet detachment from the basement membrane, and the opening of the tight junction between endothelial cells.

More recently, Butt *et al.* [19] investigated the effect of a polymerized cell-free haemoglobin (HbG) on the expression of endothelial tight junction proteins (zonula occludens 1, claudin-5, and occludin). Reduced zonula occludens 1 expression was observed after HbG transfusion as evidenced by Western blot and confocal microscopy. Claudin-5 distribution was altered in small- to medium-sized vessels.

In the present review, the endothelial junctions have been systematically analyzed in cortical biopsies of moderate and severe brain oedema associated with complicated brain trauma, congenital hydrocephalus, vascular malformation, and brain tumour in order to study their alterations, and involvement in the pathogenesis of human brain oedema. In addition to the increased transendothelial vacuolar and vesicular transport, the existence of an open interendothelial route and opening of interjunctional pockets of extracellular space of tight junctions has been demonstrated in severe oedematous areas. Emphasis has been placed upon some structural mechanisms of bypassing the endothelial junctions.

Ultrastructural alterations of endothelial junctions in moderate and severe brain oedema associated with vascular malformation

The endothelial tight junctions from capillaries of moderate brain oedema associated with vascular malformation are structurally closed and intact. The endothelial junction is visualized in its entire extent following an undulated trajectory in a plane parallel to the basement membrane, and exhibiting the intermediate dark layer that forms the quintuple-layered unit of endothelial tight junctions, as earlier described by Muir and Peter [44] (Fig. 1).

Partially open endothelial junctions are observed in collapsed brain capillaries in vascular anomalies at the level of severe oedematous regions (Fig. 2).

Pathology of endothelial junctions in traumatic brain injuries

In some moderate brain oedematous regions of traumatic brain injuries, the endothelial junctions show two zonula occludens separated by a clear non dilated extracellular space, and the luminal and abluminal ends of endothelial junctions appear to be non-enlarged (Fig. 3).

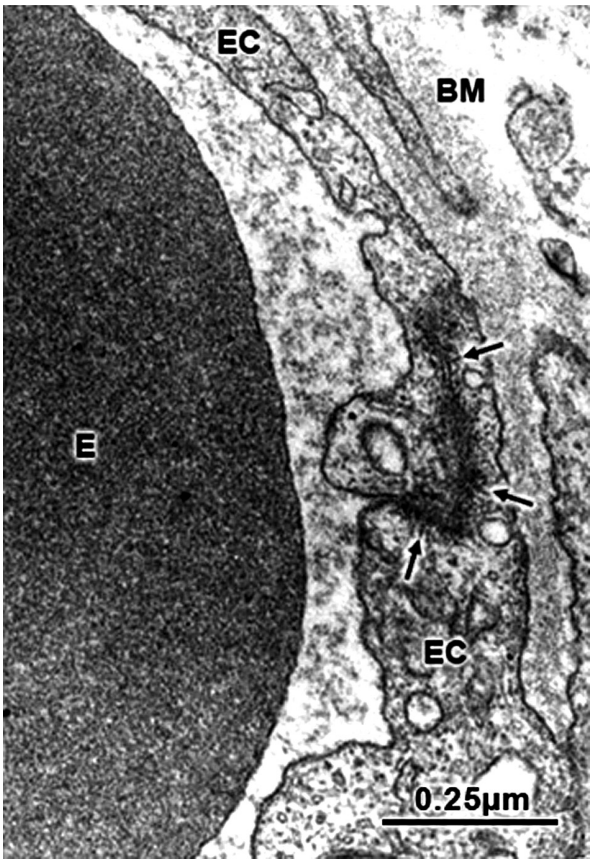

Fig. 1. Anomaly of the anterior cerebral artery. Right parietal cortex. Moderate oedematous area. A closed and intact endothelial junction exhibiting an undulated course (arrows) is connecting two endothelial cell (EC) peripheral cytoplasmic zones. An erythrocyte (E) in the capillary lumen, and the swollen capillary basement membrane (BM) are also seen.

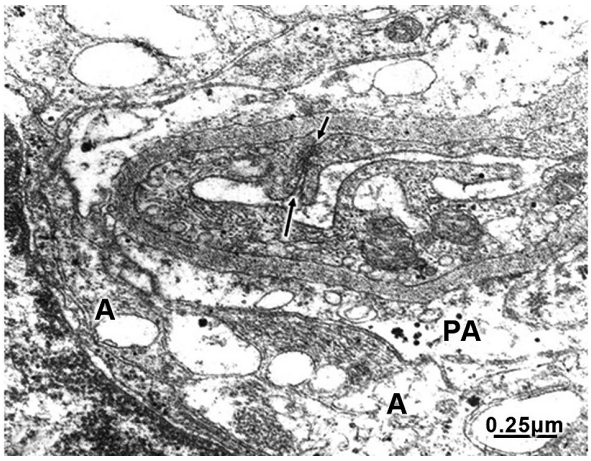

Fig. 2. Anomaly of the anterior cerebral artery. Right parietal cortex. Collapsed cortical capillary localized in a severe oedematous area. Note the partially open endothelial junction (long arrow). Some vestiges of zonula occludens are seen (short arrow). Note the notably swollen perivascular astrocyte (A), and the perivascular astrocytic end-feet (PA).

In brain trauma and perifocal moderately oedematous regions, some brain capillaries exhibit two intact and successive tight endothelial junctions (Fig. 4).

Kato *et al.* [36] also found intact endothelial junctions in rats with brain oedema and encephalopathy due to acute hepatic failure. Vaz *et al.* [76,77] reported intact tight endothelial junctions in brain microvessels of patients with traumatic cerebral contusion. Our electron microscopic images suggest that in moderate traumatic cerebral oedema, intact endothelial junctions prevent both luminal leakage and abluminal reflow from the basement membrane, supporting Broadwell *et al.* [4] earlier experiments demonstrating that tight junctions prevent the movement of proteins between endothelial cells. These findings can be correlated with the crucial and determinant role of endothelial junction proteins, such as occludin [32], claudin, ZO-1, and ZO-2 [25,27,39,78].

A consistent finding in most cases of severe traumatic brain oedema is that at the level of the parajunctional regions there is widening or dehiscence of endothelial junctions, with disappearance of the intermediate dark layer that forms the quintuple-layered unit of endothelial tight junctions described by Muir and Peter [44]. These open endothelial junctions also exhibit an undulating pathway (Fig. 5).

Apparently, partially closed intact endothelial junctions are found in the vicinity of transient transendothelial channels formed by enlarged and fused pinocytotic vesicles (Fig. 6).

On the other hand, intact endothelial junctions are found in the close neighbourhood of deep invaginations of endothelial cell peripheral cytoplasm (Fig. 7).

Goodman *et al.* [28] earlier reported loss of integrity of endothelial junctions after acute spinal cord trauma. Nakawaga *et al.* [48] found leaking endothelial junctions for water and ions following acute cerebral ischaemia after occlusion and reperfusion in rats. Bullock *et al.* [5] found disrupted tight endothelial junctions following human cerebral contusion. Mossakowsky *et al.* [43] described open endothelial junctions in global cerebral ischaemia in rats due to experimental

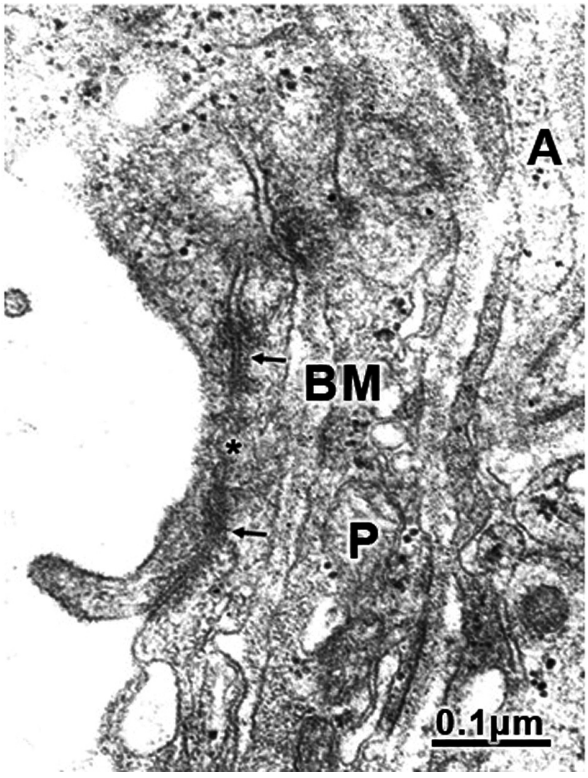

Fig. 3. Brain trauma. Subdural haematoma. Left parietal cortex. Moderate oedematous area. Intact endothelial junction showing two zonula occludens (arrows) separated by a non-dilated extracellular space (asterisk). The swollen multilayered basement membrane (BM), the enclosed pericyte (P), and the perivascular astrocytic end-foot (A) are also distinguished.

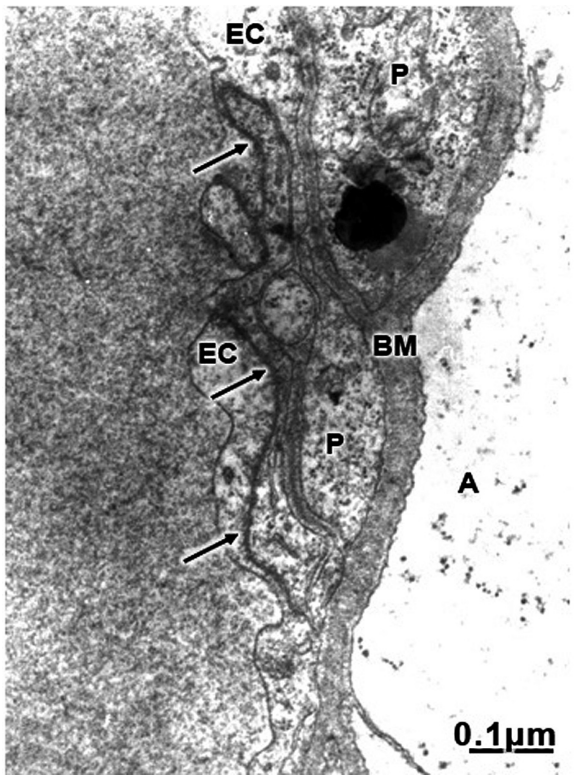

Fig. 4. Brain trauma. Right epidural haematoma. Right temporal cortex. Moderate oedematous area. Intact endothelial junctions joint neighbouring endothelial cell (EC) peripheral cytoplasmic zones (arrows) following an undulated trajectory. Note the non-oedematous basement membrane (BM) and the embedded pericyte (P). The perivascular astrocytic end-foot (A) appears to be swollen.

cardiac arrest. Urakawa *et al.* [75] reported disruption of tight endothelial junctions in brain capillaries after localized hyperthermia in rats. Besides, Baldwin *et al.* [1] demonstrated the blood-brain barrier breach after rat cortical contusions. Jaeger and Blight [34] found non-overlapping endothelial cell junctions separated by clefts after spinal cord compression injury.

Vacuoles formed in front of endothelial junctions by means of large pseudopods could empty their content through a transitory and reversible opening of the endothelial junction since the tight junction appears structurally closed or partially open [8,11]. Presumably, in this case we are dealing with a reversible opening of the blood-brain barrier induced by the brain trauma, as occurs in osmotically induced cerebrovascular permeability [3,45,60]. Physiological and ultrastructural evidence indicates that hypertonic solutions increase cerebrovascular permeability by shrinking vascular endothelial cells and widening their tight junctions [31,60]. Our patients did not receive hyperosmolar solutions in the preoperative treatment, instead they received dexamethasone. This treatment was started in order to reduce the water and sodium content of the perifocal brain oedema [62]. Steroid preoperative treatment concerns the permeability or active transport of the cellular membranes, thus exerting protective effects upon the blood-brain barrier [68]. According to Underwood *et al.* [74], glucocorticoids regulate transendothelial fluid flow resistance and formation of endothelial junctions. Therefore, we assume that in traumatic brain injuries, the shrunken endothelial peripheral cytoplasm and open endothelial junctions are mainly due to mechanical forces acting upon endothelial junctions. It is also known from other non-

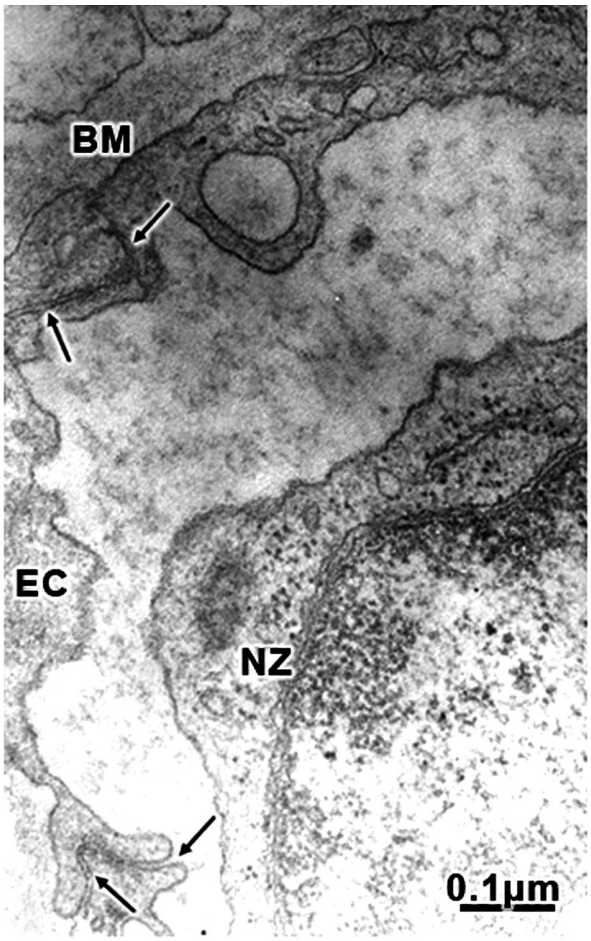

Fig. 5. Brain trauma. Right parieto-temporal haematoma. Right parietal cortex. Severe oedema. Brain capillary showing two open endothelial junctions (arrows). Note the disappearance of zonula occludens and the enlarged extracellular space separating the two neighbouring endothelial cells (EC). Note the swollen basement membrane (BM). The prominent endothelial cell nuclear zone (NZ) is also seen.

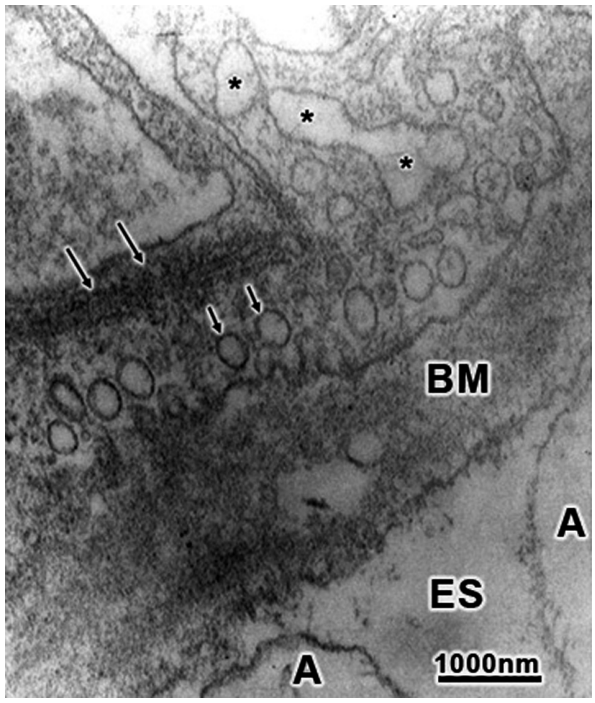

Fig. 6. Brain trauma. Subdural haematoma. Left parietal cortex. Partially closed endothelial junction (long arrows) in the vicinity of an incomplete transendothelial channel formed by fusion of elongated vacuoles (asterisks) extending from the endothelial cell luminal plasma membrane to the neighbouring capillary basement membrane (BM). Note the numerous pinocytotic vesicles (short arrows) in close proximity to the swollen and vacuolated capillary basement membrane (BM). The perivascular astrocytic end-feet (A) appear dissociated (arrow) from the basement membrane, and separated by the enlarged extracellular space (ES).

nervous tissues that tight junctions between many epithelial cells are deformed by osmotic and hydrostatic pressure stresses that simultaneously increase junctional permeability [55]. Opening of tight junctions has also been reported in acute hypertension produced by a pressure-pulse wave technique [46]. Dehiscence of undulated endothelial junctions has been earlier observed by Griffiths [29] after impact injury and by Garcia *et al.* [24] in ischaemic injuries.

According to earlier observations of Povlishock *et al.* [59], hypertension and brain trauma never elicited tight junctional cleaving. However, the above-mentioned authors considered acute events only. In our study, the evolution time of the brain injury varied from 24 hours to two years, and in patients with a long evolution time of brain injury, the associated ischaemic process of traumatic lesions contributes to the endothelial cleft alteration.

In patients with brain trauma and subdural haematoma, with tonic-clonic convulsions, loss of consciousness and behavioural disorders, we found in very severely traumatic oedematous areas, open endothelial junctions with marked enlargement of interjunctional pockets of extracellular space. In addition, the endothelial junctions offer a beaded aspect [7,14]. Apparently

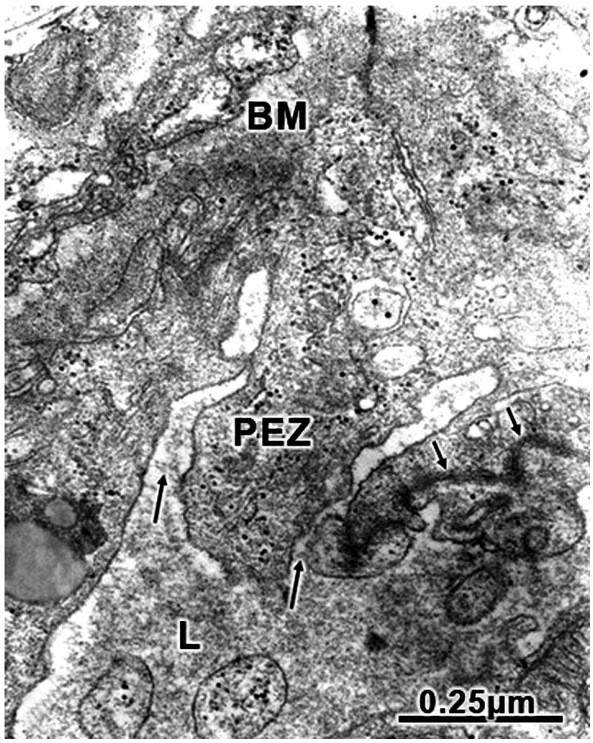

Fig. 7. Brain trauma. Subdural hygroma. Left parietal cortex. Deep and parallel invaginations of luminal endothelial plasma membrane of endothelial cell peripheral zone (long arrows) are seen extending toward the vicinity of capillary basement membrane (BM). The endothelial junction (short arrows) appears to be structurally intact. The capillary lumen (L) and the swollen basement membrane (BM) are also distinguished.

there is an increased intercellular passage of haematogenous oedema fluid through these interjunctional pockets. Chemical and electrically induced seizures have been found to increase the permeability across cerebral vessels [80]. Presumably, brain trauma and convulsions simultaneously and reciprocally influence brain capillary permeability through opening of endothelial junctions [14]. These interjunctional pools apparently enable the entry of haematogenous oedema fluid, which could osmotically induce opening of tight junctions, in a way similar to that described by Brightman *et al.* [3] using hyperosmotic 3M urea. In the parajunctional areas, increased vesicular and vacuolar transport and formation of transendothelial channels are also found [8,9], suggesting that in highly increased human cerebrovascular permeability there are possibly two main routes: a transendothelial conduit (vacuolar and vesicular transport, transendothelial channels) and an interendothelial pathway (highly leaky endothelial junctions), accounting for capillary leakage. These pathways would be undoubtedly relevant mechanisms operating in the genesis of vasogenic human brain oedema.

In severe brain oedema, some mechanisms such as: communicating pits with the extracellular space at the basal segment of the endothelial junctions, chained micropinocytotic vesicles between the endothelial luminal membrane and the basal segment of endothelial junction, and tubular structures linking the extracellular pockets of endothelial junctions with basement membrane bifurcation are found for bypassing the endothelial junctions [10]. Such bypassing mechanisms strongly suggest that even in severe oedema, the tight junctions prevent the luminal passage of oedematous fluid, and that these shuttle connections account for the enhanced intercellular passage at the level of the basal region of endothelial junctions. The vesicular transport can be diverted to the extracellular space or junctional extracellular pockets separating the apposed endothelial membranes at the level of the basal region of endothelial junctions. From this intercellular space the haematogenous fluid would be channelled to the basement membrane. In this manner the vesicular transport bypasses the tight endothelial junctions.

In severe traumatic oedematous regions, there is notable dilation of the basal segment of endothelial junction, whereas the luminal segment provided with tight junctions is apparently intact, suggesting that there is no retrograde passage of oedema fluid through the tight junctions. In these cases, the basement membrane bifurcations are considerably dilated and act apparently like capillary abluminal channels. In addition, tubular structures connecting the extracellular pockets of endothelial junctions with the basement membrane bifurcations are encountered in traumatic brain oedema of long evolution time. These tubular structures seem to be pathways for facilitated transport between the extracellular space and the basement membrane [12].

That tight junctional cleaving or open junctions are not artefacts of immersion-fixed cortical biopsies used for transmission electron microscopy is attested by the fact that open clefts are observed only in capillaries located in very severe oedematous areas with the presence of enlarged extracellular spaces in the

perivascular neuropil. For ethical reasons it is impossible to obtain controls of 'normal' human tissue. In addition, we have to bear in mind that normal human tissue is difficult to observe since once the craniotomy is performed, and the brain surface is exposed to air, the brain oedema begins to appear, as earlier demonstrated by Prados *et al.* [56].

Endothelial junction derangement in congenital hydrocephalus

In congenital hydrocephalus, the capillary wall shows evident signs of the blood-brain barrier dysfunction characterized by increased endothelial vesicular and vacuolar transport, closed and open interendothelial junctions, thin and fragmented basement membrane with areas of focal thickening, and discontinuous perivascular astrocytic end-feet [16] (Fig. 8).

In severely oedematous areas featured by a disrupted neuropil, the endothelial junctions appear partially open at both the luminal and abluminal sites. In addition, the capillaries exhibit endothelial cell increased vesicular and vacuolar transendothelial transport, and swollen and disrupted basement membrane (Fig. 9).

Our findings in human hydrocephalus favour the idea of an interendothelial route either for oedema formation or resolution in human hydrocephalic cerebral cortex [15-17]. Nakagawa *et al.* [49] also reported widening of interendothelial clefts between the tight junctions in capillaries of the subependymal and subcortical white matter in hydrocephalic rats, and postulated the possibility of a paracellular route for hydrocephalic oedema resolution. In this context, human hydrocephalus differs from the hy-3 mouse neonatal hydrocephalus [42], in which a normal operating blood-brain barrier is suggested by the presence of intact interendothelial tight junctions, no indication of increased pinocytotic activity, and perivascular astrocytes of apparently normal submicroscopic morphology.

Okuyama *et al.* [51] have also reported a disturbed microcirculation in congenitally hydrocephalic brain of the inbred rats LEW/Jms, especially at the level of endothelial cells of capillaries and venules in the periventricular oedematous region.

Tight endothelial junction opening in peritumoural oedema

Capillaries examined in severely perifocal oedematous areas surrounding brain tumours show open endothelial junctions. In this region, abundant proteinaceous oedema fluid in the perivascular space is observed (Fig. 10).

Shibata *et al.* [70,71] earlier described open endothelial junctions in cerebral oedema associated with glioblastoma, astrocytoma and meningioma. Castejón [8,11] showed open endothelial junctions in capillaries of peritumoural oedema surrounding craniopharyngioma. Papadopouslo *et al.* [53] and Davies [20] described open endothelial junctions in high grade astrocytomas, which exhibited loss of occludin expression. Liebner *et al.* [40] reported the loss of expression of claudin-1 in the majority of vessels of human glioblastoma multiforme, related with the increased endothelial cell permeability. Roy and Sarkar [65] reported partially or completely opened-up junction in human brain tumour micro blood vessels (MBVs).

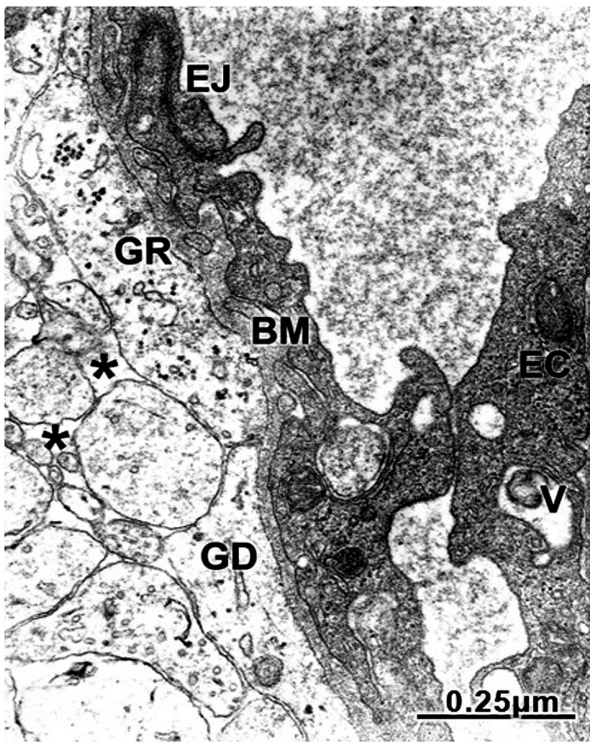

Fig. 8. Arnold-Chiari malformation and congenital hydrocephalus. Moderate oedema. Right frontal cortex. Longitudinally sectioned capillary showing an apparently intact and undulated tight junction (EJ), endothelial cell vacuoles (V), and the thin and swollen capillary basement membrane (BM). The swollen perivascular glycogen rich (GR) and glycogen depleted (GD) astrocytic end-feet, appear attached to the basement membrane. Note the enlarged hydrocephalic extracellular space (asterisks).

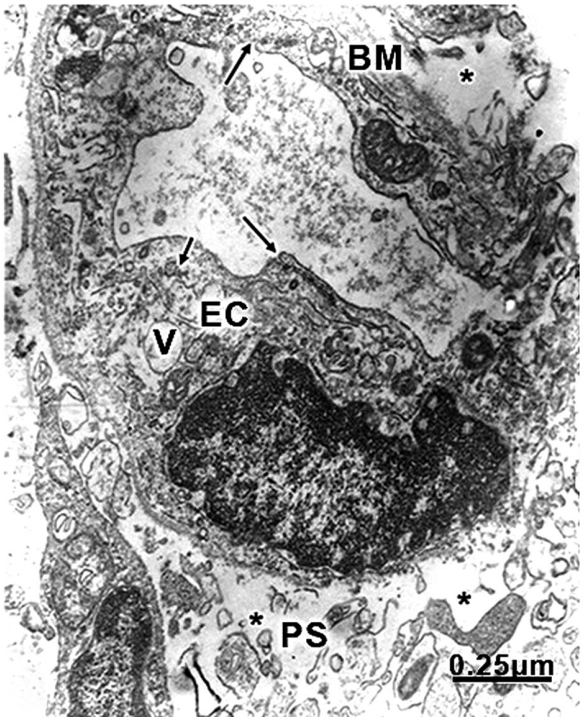

Fig. 9. Congenital hydrocephalus. Arnold-Chiari malformation. Right parietal cortex. Isolated capillary located in a disrupted neuropil in a severely oedematous area. The endothelial junction (long arrows) appears to be partially open. The endothelial cell (EC) shows vacuoles (V), and dense inclusion bodies (short arrow). Note thin and fragmented capillary basement membrane (BM), and the enlarged perivascular space (PS, asterisks).

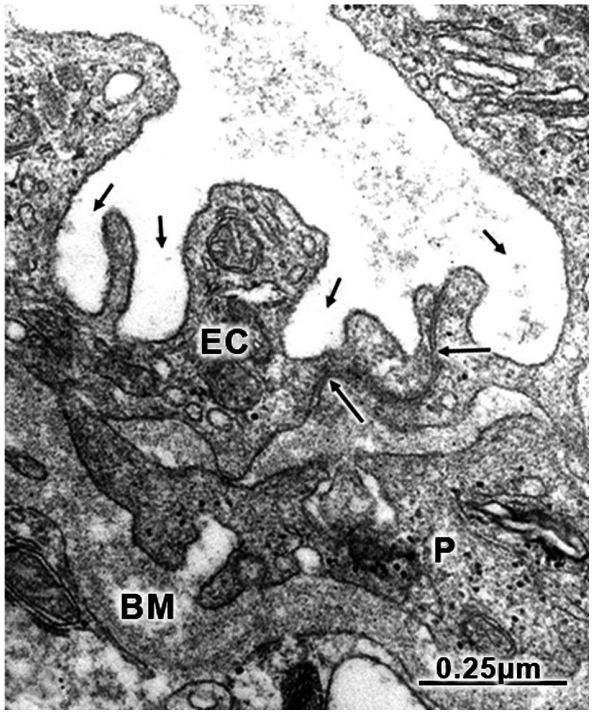

Fig. 10. Cystic craniopharyngioma. Right temporal cortex. Open endothelial junction following an undulating course (long arrows). The short arrows indicate the deep invaginations of peripheral endothelial cytoplasm (EC). Note the extremely swollen basement membrane (BM), and the enclosed oedematous pericyte (P).

The significance of junctional dehiscence in the pathogenesis of human brain oedema remains to be elucidated. Several possibilities should be considered: a) mechanical cleavage of endothelial tight junction by the traumatic injury and the ischaemia associated with the chronic state of the pathological process [8,11]; b) serotonin or histamine release in brain trauma, which induces endothelial cell contraction and opening of endothelial junctions [80]; c) increased elevation of intracellular c-AMP [81]; d) osmotic disruption due to solute and plasma protein movement into the compartments of endothelial tight junction and the subsequent movement of water [3,45]; e) alteration of actin cytoskeleton architecture [10]; f) post-translational modification of junctional proteins [18,32,40]; g) proteolytic degradation of junctional constituents [41]; h) effect of proinflammatory cytokines [72], and haemoglobin, iron deposition, and oxidative end products [19].

The osmotic gradient would produce focal discontinuities in the junctional protein fibril [46], while the osmotic disruption would induce interendothelial cleft dehiscence. According to Fischer *et al.* [21-23], and Mark and Davis [41], hypoxia disrupt the continuity of the tight junction protein zonula occludens-1 (ZO-1). On the other hand, Hamm *et al.* [30] considered that opening of tight junctions is not accompanied by any change in the molecular composition of endothelial junction. Nagy *et al.* [47] reported the opening of paracellular avenues in human microvessel endothelial cell culture in ischemic/hypoxic conditions.

Concluding remarks

Cortical biopsies of patients with the diagnosis of complicated brain trauma, congenital hydrocephalus, brain vascular anomaly, and brain tumour are studied with the electron microscope using cortical biopsies

of different cortical brain regions to analyze the alterations of endothelial junctions, and their participation in the pathogenesis of human brain oedema. In moderate oedema, most endothelial tight junctions are structurally closed and intact, while in some cases of severe oedema, the opening of tight endothelial junctions is observed. In very severe brain oedema, a considerable enlargement of interjunctional pockets of extracellular space is also seen suggesting that in highly increased cerebrovascular permeability, the endothelial junctions are open in their entire extent, and that an intercellular or paracellular route through interendothelial clefts for transferring haematogenous oedema fluid from blood to the capillary basement membrane and the brain parenchyma is formed, contributing to the formation of brain oedema.

Some accessory mechanisms of bypassing the endothelial junctions are also found, such as: a) communicating micropinocytotic vesicles with the extracellular space at the basal segment of endothelial junction; b) chained micropinocytotic vesicles between endothelial luminal membrane and basal segment of endothelial junction and c) tubular structures connecting the extracellular pockets of endothelial junction with the basement membrane bifurcation. High intensity brain trauma, seizures, and osmotic forces would explain the opening of endothelial junctions in severe and complicated brain oedema.

In congenital hydrocephalus, the capillary wall shows evident signs of the blood-brain barrier dysfunction characterized by closed and open interendothelial junctions, increased endothelial vesicular and vacuolar transport, thin and fragmented basement membrane with areas of focal thickening, and discontinuous perivascular astrocytic end-feet. The perivascular space is notably dilated and widely communicated with the enlarged extracellular space in the neuropil, showing the contribution of damaged endothelial junction to the formation of interstitial or hydrocephalic brain oedema.

Acknowledgements

This paper has been prepared owing to a subsidy obtained from CONDES-LUZ, and Fundadesarrollo-LUZ.

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