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Ultrastructural pathology of cortical capillary pericytes in human traumatic brain oedema

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Abstract

In human traumatic brain oedema pericytes exhibit remarkable oedematous changes, increased vacuolar and vesicular transport, transient transpericytal channels, and tubular structures demonstrating pericyte brain barrier dysfunction. They show nuclear invaginations, actin and myosin-like filaments, and coupled interaction with endothelial cells through the macula occludens. Some pericytes display hypertrophic and necrotic changes, and phagocytic capacity. Hypertrophic pericytes induce basement membrane splitting. Degenerated pericytes exhibit lacunar enlargement of endoplasmic reticulum, dense osmiophilic bodies, glycogen granules, vacuolization, oedematous Golgi apparatus, and pleomorphic mitochondria. Certain micropinocytotic vesicles are orientated to the Golgi complex and multivesicular bodies, suggesting that pericytes play some role in oedema resolution.

Key words: *pericytes, brain oedema, brain trauma, cerebral cortex, electron microscopy.*

Introduction

The behaviour of pericytes has been explored under experimental conditions and in some neuropathological states [1,8,13,30,44-46,55,67]. Brierley and Brown [6] described pericyte degeneration, but not phagocytic activity, in cerebral infarct. Semchenko *et al.* [62] found poorly metabolized metabolites in the pericytal cytoplasm in patients with brain tumours. Castejón [10] described the ultrastructural changes of pericytes in human brain oedema associated with congenital malformations, brain trauma, and brain tumours. Jeynes [31] described an increased number of acid phosphatase positive granular pericytes with accumulating lipid components after ischaemic insult. Liu [42] reported active and proliferative activities of pericytes in the neovasculature in ischaemic brain infarct. Herman and Jacobson [28] confirmed the presence of pericyte filament-enriched processes in hypertensive rat brains, and suggested an important role of pericytes in hypertension and cerebrovascular diseases. Glees *et al.* [21] described oedematous hypertrophic pericytes in hydrocephalic human infants demonstrating brain-barrier dysfunction. Schlingemann *et al.* [61] found an increased number of pericytes positively stained with human high molecular weight-melanoma associated antigen (HMW-MAA), in conditions associated

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with vascular proliferation in tumours and healing wounds. Liwnicz *et al.* [40] showed pericyte degeneration and thickening of basement membrane of cerebral microvessels in intractable complex partial seizures. Tagami *et al.* [66,67] observed granular and filamentous pericytes in stroke-prone spontaneously hypertensive rats. These authors postulated the granular pericytes as scavenger cells, and found filamentous pericyte degeneration during development of hypertension.

Wegiel and Wisniewski [74] reported the presence of tubulo-reticular structures in pericytes in brain biopsies of patients with Alzheimer's disease. According to Perimutter [53], pericytes have been implicated in vascular alterations and cerebrovascular amyloid deposition in Alzheimer's disease. Robinson *et al.* [59] found proliferation of pericytes (pericytosis) in a distinctive variant of meningioma associated with severe peritumoural oedema. Verbeek *et al.* [72] found rapid degeneration of cultured human brain pericytes with increased production of cellular amyloid precursor beta protein. Bertossi *et al.* [5] reported pinocytotic vesicles and phagocytic bodies in pericytes of peritumoural capillaries. Popova and Zagrebina [57] described destructive changes of pericytes in atherosclerotic dementia.

Verbeek *et al.* [73] studied the relation between the amyloid-beta induced degeneration of human brain pericytes with the apolipoprotein E genotype. Frontczak-Baniewicz *et al.* [20] presented evidence of pericyte migration through the capillary basement membrane in rat focal brain compression. Dore-Duffy *et al.* [15] reported similar findings in rat traumatic brain injury. According to these authors, non-migrating pericytes showed rapid degenerative changes. Lupo *et al.* [42] described pericyte shrinkage of the cell body, retraction of processes, and disruption of the intracellular actin network induced by in vitro amyloid beta incubation of retina capillaries.

Gonul *et al.* [22] found an early pericyte response and migration to brain hypoxia in cats. Hayashi *et al.* [20], studying the effects of hypoxia on an endothelial/pericytic co-cultured model of the blood-brain barrier, considered that pericytes affect the endothelial cells by secreting factors or through a gap junction. Melgar *et al.* [50] found detachment and migration of pericytes in an awake model of transient forebrain ischaemia in rats. Yamaghisi and Imaizumi [75] described the pathological role of pericyte loss or dysfunction in various devastating disorders such as diabetic retinopathy, atherosclerosis and tumour angiogenesis. Dore-Duffy *et al.* [16] and De Gracia *et al.* [11] found TUNEL positive pericyte cell death following animal traumatic brain injury. Li *et al.* [38] postulated a key role of pericytes in vascular remodelling, and in the pathogenesis of vascular malformations.

Hayden *et al.* [22] pointed to the possibility of the pericyte cell being one of many contributors to the fibrogenic pool of cells important for peri-islet fibrosis as a result of excess angiotensin II at the local tissue level in the Ren2 rat model of hypertension.

Typical changes of the pericytes featuring accumulation of lipofuscin-like material and their degeneration were reported by Szpak *et al.* [64] in familial amyloid and non-amyloid angiopathies. Ammoury *et al.* [3] reported abundant electron-dense membrane-bound granules in pericytes in a patient with photoexposed hyperpigmented skin after amiodarone treatment. Hayden *et al.* [22-24] demonstrated significant pericapillary amyloid deposition and diminution of pericyte foot processes in pericytes in the HIP rat model of diabetes.

According to Hayden *et al.* [23], hypercellularity consisting of pericytes and inflammatory cells is observed in T2DM pancreatic tissue. Organized fibrillar collagen was closely associated with pericytes, which are known to differentiate into myofibroblast-pancreatic stellate cells.

Piquer-Gil *et al.* [56] provided direct evidence that the cell fusion process contributes to the formation of pericytes after stroke. In mice, the authors detected X-gal-positive cells that expressed vimentin and desmin, specific markers of mature murine pericytes. They concluded that cell fusion participates actively in the generation of vascular tissue through pericyte formation under normal as well as pathological conditions.

Shi [63] demonstrated that cochlear pericytes are markedly affected by acoustic trauma and displayed an abnormal morphology and lost their tight association with endothelial cells. The author demonstrated that the levels of the pericyte structural protein desmin substantially increased after noise exposure in both guinea pigs and mice, with a corresponding increase in pericyte coverage of vessels.

Li *et al.* [39] described narrow or occluded blood vessels sometimes with contracted endothelial cells

and pericytes in malignant breast tumours. Pavlov *et al.* [53] reported a reduced count of pericytes in peripheral arteriovenous and venous angiodysplasias.

Van der Avoort *et al.* [70] found by means of electron microscopy detachment of pericytes from vascular endothelial cells in lichen sclerosus for vulvar squamous cell carcinoma.

Gerrits *et al.* [25] recently described that about 70% of the pericytes contained degenerative inclusions in changes in oestrogen-α sensitive brainstem structures of aging female hamsters. Lewandowska *et al.* [37] observed the reduction and loss of pericytes in capillary vessel wall in CADASIL angiopathy. According to Medrado *et al.* [49], low level laser therapy induced the proliferation and migration of pericytes to the extracellular matrix and their phenotypic modulation to myofibroblasts during tissue repair during experimental skin wound healing in Wistar rats.

Fisher *et al.* [18] demonstrated in selected cases, by means of electron microscopy, pericyte involvement in cerebral microbleeds in the elderly.

The present review is devoted to examining the pericyte swollen and degenerative changes, reactive response, phagocytic activity, contractile properties, and blood-barrier dysfunction involvement in human and complicated traumatic brain injuries. In this context, traumatic human brain oedema constitutes an excellent model to study pericyte blood-brain barrier involvement. Attention is therefore focused on the pericytal mechanisms in enhanced cerebrovascular permeability. The comparative behaviour of the endothelial cell-pericyte unit is also described, mainly in relation to transcapillary exchange and capillary contractility.

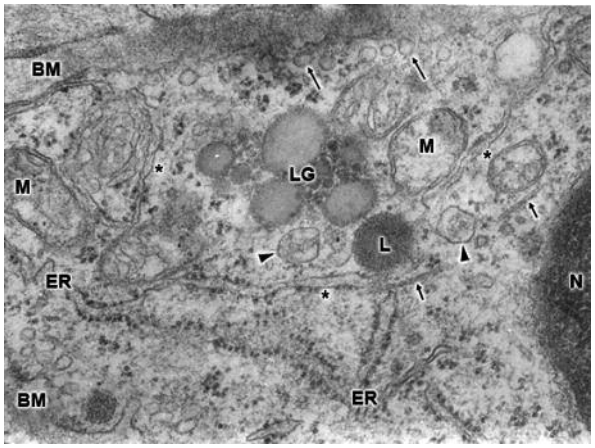

Fig. 1. Pericyte cell from a moderate oedematous region showing increased micropinocytotic transport (arrows) beneath the basement membrane (BM) and lipid granules (LG) deposition. The well-developed rough endoplasmic reticulum (ER) appears slightly dilated. Swollen mitochondria (M), microtubules (small arrows), primary lysosomes (L), and protein-containing vacuoles (arrowheads) are also seen. The nucleus (N) with dense heterochromatin is visualized on the right side of the figure.

Pericyte morphological changes in moderate and severe traumatic brain oedema

Oedematous pericytes

In capillaries localized in moderate oedematous traumatic areas, the pericytes, always enclosed by a thickened basement membrane, exhibit increased hypolemmal micropinocytotic transport, slightly dilated rough endoplasmic reticulum, and moderate hydropic changes of the cytoplasmic matrix. The mitochondria also show oedematous changes of their matrix and cristae. Additionally, lipid droplets, primary and secondary lysosomes, small protein-containing vacuoles, coated vesicles and clear and dark microtubules are found (Fig. 1).

In severe oedematous areas, where structural damage of the basement membrane is encountered, a remarkable pericytal swelling occurs, characterized by lacunar enlargement of rough endoplasmic reticulum containing haematogenous oedema fluid. There is also an increased number of pleomorphic and swollen mitochondria, and deposition of large lipid droplets. In these oedematous pericytes the rough endoplasmic reticulum canaliculi appear as an enlarged prominent circulatory system connecting the basement membrane with the perinuclear cistern (Fig. 2).

Some micropinocytotic vesicles appear connected to the rough endoplasmic reticulum canaliculi, apparently discharging their content into the lumen of the endoplasmic reticulum. This orientated transport toward the endoplasmic reticulum partially explains the subsequent lacunar enlargement of endoplasmic cisterns, which appear to contain proteinaceous oedema fluid.

Oedematous pericytes have also been earlier reported in delayed radionecrosis of the brain by McDonald and Hayes [48]. Conversely, in anoxic-

ischaemic lesions Hills [30] did not report pericyte oedema, and supposed that these cells possess different metabolic characteristics from the endothelium, and are capable of a greater degree of anaerobic independence. Also, extensive intrapericytic oedema was observed by Dodson *et al.* [13,14] in animals with longer periods of ischaemia, presumably due to sustained anoxic-ischaemic lesions.

Pericyte degeneration

In very severely oedematous areas where haematogenous oedema fluid is present in enlarged neuropile extracellular spaces and notable thickening of the basement membrane is seen, the pericyte cells suffer degenerative changes characterized by discontinuous plasma membrane, wide communications between the pericytal cytoplasm, damaged basement membrane matrix, hydropic changes of the Golgi apparatus and vacuolization [9,10].

As depicted in Fig. 3, areas of rarefaction with focal necrosis of pericyte cytoplasmic matrix are found. Due to these alterations, these cells have been considered as degenerated pericytes. These findings show that in traumatic brain oedema pericytes progressively lose their barrier function and develop intrinsic hydropic changes leading to pericyte necrotic areas.

Degenerative changes of pericytes have also been reported by Brierley and Brown [6], and Liwnicz *et al.* [40] after intractable complex partial seizures, and by Verbeek *et al.* [72,73], Lupo *et al.* [42], and Rensink *et al.* [58] following amyloid beta deposition.

Hypertrophic pericytes

After a traumatic injury of long evolution time, the pericyte may exhibit hypertrophic changes characterized by an increased amount of endoplasmic reticulum, which exhibits a labyrinthine aspect, and voluminous processes, which induce basement membrane splitting [10]. Hypertrophy of some peri-

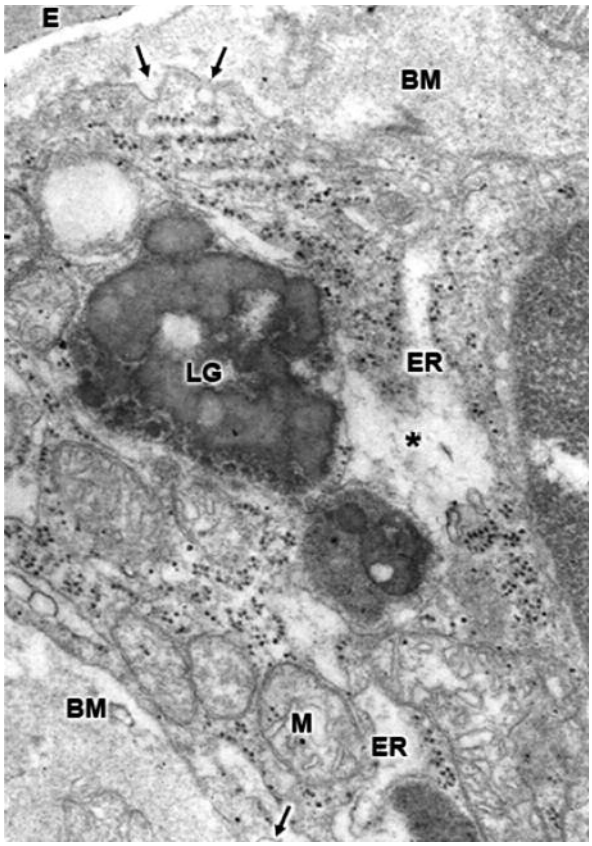

Fig. 2. Pericyte from a severely oedematous area showing increased micropinocytotic transport (arrows) beneath the extremely swollen basement membrane (BM) directed toward the rough endoplasmic reticulum (ER), which appears as a remarkably dilated lacunar system (asterisk). Large lipid granules (LG) and microtubules (arrowhead) are visualized. Note the increased number of pleomorphic and swollen mitochondria (M).

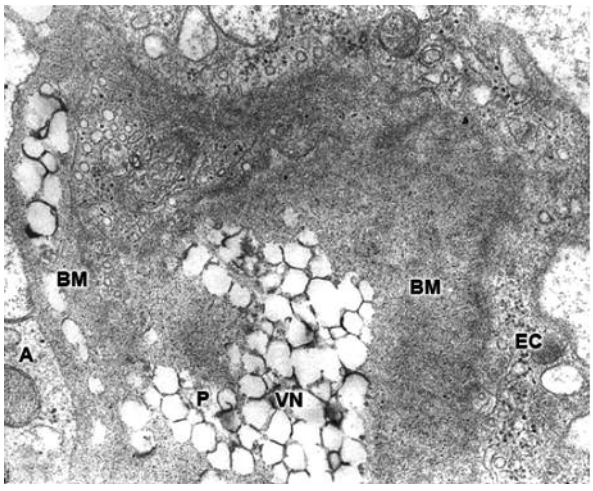

Fig. 3. Brain trauma. Left parieto-occipital subdural hygroma. Left temporal cortex. Pericytal process (P) showing extensive vacuolar necrosis (VN). The vacuolization extends to the basement membrane (BM). Note the endothelial cell (EC) and the swollen astrocytic end-feet (A).

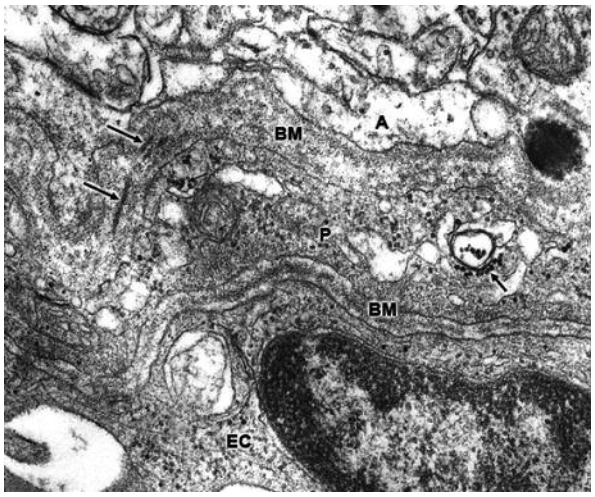

Fig. 4. Brain trauma. Right parieto-temporal subdural haematoma. Right parietal cortex. Pericytal process (P) showing vacuolization (V) and increased amount of glycogen granules (short arrow). The basement membrane (BM) exhibits proliferation of collagen fibres (long arrow). The endothelial cell (EC) nuclear zone and the astrocytic end-feet (A) are also visualized.

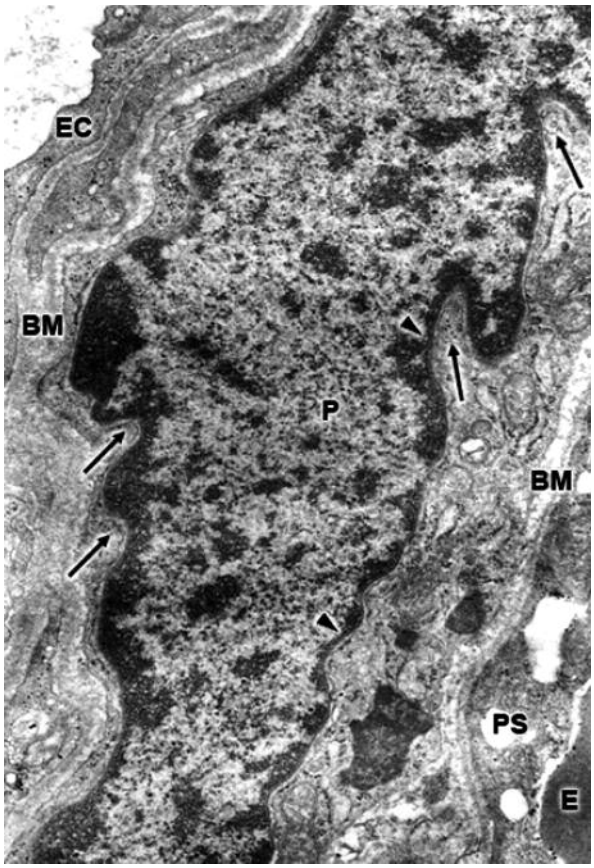

Fig. 5. Brain trauma. Left fronto-parietal-occipital haematoma. Left parietal cortex. Contracted pericyte (P) enclosed by the basement membrane (BM) exhibiting the characteristic nuclear invaginations (long arrows). The nuclear dense lamina (arrowheads) can be seen between the perinuclear cistern and the peripheral heterochromatin, and appear interrupted at the nuclear pores (small arrows). The endothelial cell (EC) is visualized at the upper left hand corner of the figure. An extravasated erythrocyte (E) is observed in the perivascular space, which shows haematogenous dense fluid (PS).

cytes was observed by Maxwell and Kruger [46] as a limited reactive response following low doses of irradiation. Pericyte hypertrophic changes have also been reported by Markov and Dimova [44] in chronic poisoning, and by Glees *et al.* [21] in hydrocephalic human infants. Additionally, simultaneous vacuolization and deposition of glycogen granules are observed in the pericytal cytoplasm. The presence of an increased amount of glycogen granules in pericytes as herein observed in traumatic brain oedema is an unusual finding (Fig. 4).

Glycogen granules have been observed in small amounts in brain pericytes [46] following brain irradiation, and have not been seen even in related cells such as microglial cells [52]. The abnormal deposition of glycogen in pericytes presumably reflects poor oxygen consumption or a high rate of anaerobic metabolism due to the brain trauma and perifocal brain oedema.

We have not found evidence of transformation of pericytes into microglial cells, for example, images of a pericyte in the course of separation from the vascular wall as described in experimental animal studies [33,51,65,71]. Similarly, Dodson *et al.* [14] do not report transformation of pericytes into phagocytes in cerebral ischaemia. It has been postulated that pericytes may divide and send off daughter cells into the nervous tissue, where they become either macrophages [46] or activated microglia [7,33]. Pericyte migration was earlier denied by Kitamura [33] in traumatic brain lesions. However, more recent investigations have demonstrated pericyte migration in traumatic brain injuries [15], and after focal brain compression [20] as an early response to hypoxia [26], and following an awake model of transient forebrain ischaemia [50].

Pericyte contractile activity

Pericytes displaying a contracted shape are encountered characterized by numerous deep and shallow invaginations of the nuclear envelope forming notches and folds. In these nuclei the dense basal nuclear lamina is clearly distinguished beneath the inner nuclear membrane, disclosing the pericyte mesodermal origin (Fig. 5).

According to Desaki and Nishida [12], the constriction and/or contraction of microvessels by smooth muscle cells, and degenerated pericytes may be involved in the degeneration and remodelling of the microvascular network in the muscle bundles following degeneration and regeneration of the muscle fibres.

Pericyte endothelial cell interaction

The pericytal processes are coupled to the endothelial peripheral cytoplasm by means of typical macula occludens. At this level, the basement membrane separating both cells disappears and their plasma membranes become fused (Fig. 6).

These macula occludens presumably represent specialized electrical contact sites, where the excitability of contracted pericytes can be transmitted to the neighbouring endothelial cells.

The contractile properties of pericytes were formerly postulated by Rouget [60] as a capillary sphincter action. The pericytes were regarded by Farquhar and Hartmann [17], and Maynard *et al.* [47] as primitive or modified smooth muscle cells. The contracted pericytes exhibit similar features to those reported by Majno *et al.* [43] in endothelial cell contraction induced by histamine-type mediators. Presumably, pericyte contraction can be transmitted through the macula occludens existing between endothelial cells and pericytes. This coupled cell interaction could be responsible for capillary sphincter function, as earlier postulated by Rouget [60], and could also be a relevant mechanism in relation to clinical symptoms of vasospasm and vascular headache. The microfilaments encountered in the pericytal cytoplasm, and identified as actin-like and myosin-like filaments [35,36], are involved in contractile activity. Also, actin and myosin have been demonstrated in pericytes by immunohistochemical methods [52]. Since some fine actin-like filaments are observed attached to the pericytal plasma membrane and the basement membrane surface, it seems plausible that these fine filaments also influence the activities of the pericyte surface and basal lamina. Pericyte contractility has also been reported by Stensaas [65] in the basal forebrain of neonatal rabbits, and Herman *et al.* [29] in normotensive and hypertensive rat brain, and is considered to play a pivotal role in regulating the blood flow within the brain microcirculation [70].

Hermann *et al.* [30] examined pericyte-endothelial cell interaction in vitro, and found pericytes rich in muscle and non-muscle actin. Allt and Lawrenson [2] emphasized the interaction of pericytes and endothelial cells and its importance for maturation, remodelling and maintenance of the vascular system via the secretion of growth factors, modulation of extracellular matrix, and regulation of vascular permeability. Hayashi *et al.* [27], studying the effects of hypoxia on an endothelial/pericytic co-cultured model of the blood-brain barrier, considered that pericytes affect the endothelial cells by secreting factors or through a gap junction.

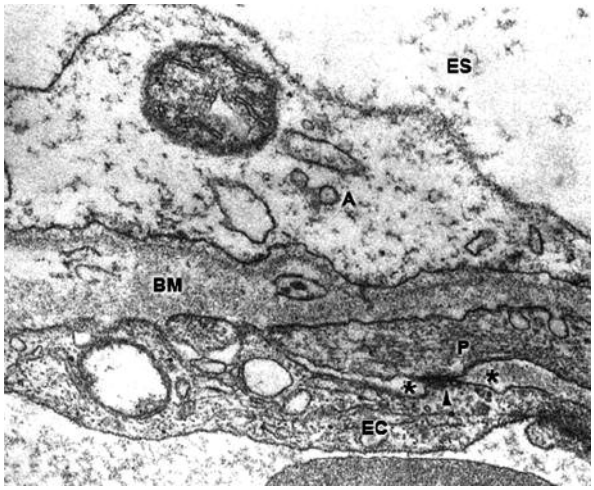

Fig. 6. Brain trauma. Right epidural haematoma. Right temporal cortex. Pericytal process (P) embedded into the basement membrane (BM) coupled with the endothelial peripheral cytoplasm (EC) by means of a macula occludens (arrowhead). The basement membrane expansions (asterisks) separating both cells disappear at this level. The endothelial cell (EC), the swollen perivascular astrocytic end-foot (A), and the enlarged extracellular space (ES) are also seen.

Dense and clear microtubules appear randomly dispersed throughout the pericyte cytoplasmic body and processes. Our findings tend to favour the idea that the cytoskeleton is also involved in pericyte contraction and enhanced micropinocytotic transport [10].

The pericyte cell role in blood brain barrier function

Pericytes are accepted as responsible for some facets of the blood-brain barrier [14], which emphasize the importance of studying the role of pericytes in the blood-brain barrier system. In traumatic brain injury of short evolution time (24 h), with severe cerebral oedema, the pericytes show, like the endothelial cells, increased vesicular and vacuolar transport, revealing loss of the barrier function of both cells (Fig. 7).

In severe brain oedema of long evolution time, pericytes show elongated micropinocytotic vesicles forming transient transpericytal channels originating from a combined process of membrane fusion and fission. Some of these channels appear tortuous, dilated and directed toward the rough and smooth endoplasmic reticulum canaliculi (Figs. 8 and 9). Tubular structures connecting the pericytal

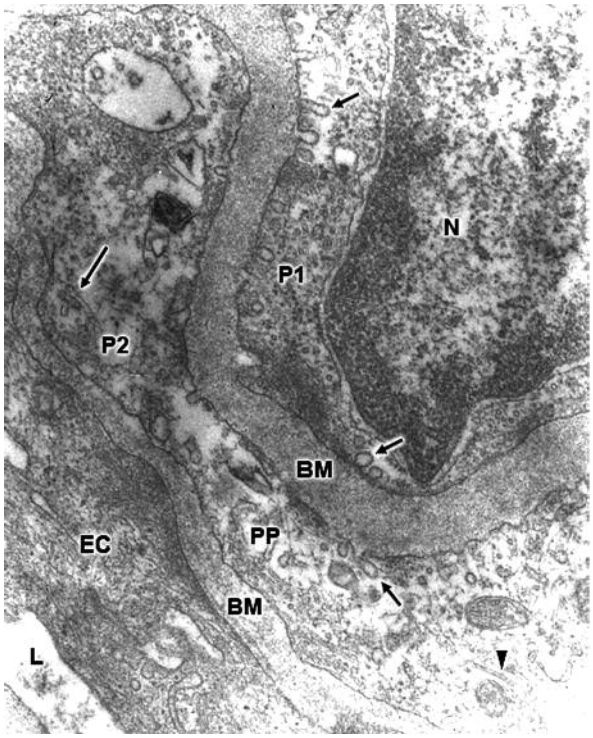

Fig. 7. Brain trauma. Frontal contusion. Left frontal cortex. Two overlapping oedematous pericytes (P1 and P2), separated by the basement membrane (BM), appear showing respectively the nuclear region (N) and the peripheral process (PP). They appear notably oedematous and exhibit increased pinocytotic transport (small arrows). Actin-like filaments (long arrow) and microtubules (arrowheads) are also visualized. Note the endothelial cell (EC), and the capillary lumen (L).

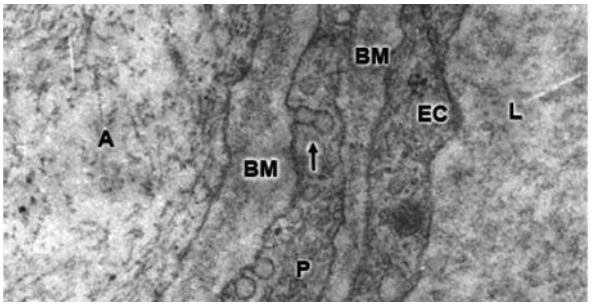

Fig. 8. Brain trauma. Right parieto-temporal subdural haematoma. Right parietal cortex. Pericytal process (P) showing chained pinocytotic vesicles (arrow) extended almost through its entire width and tending to form a transpericytal channel between the basement membrane (BM) division. The perivascular astrocyte cytoplasm (A), endothelial cell (E) and capillary lumen (L) are also distinguished.

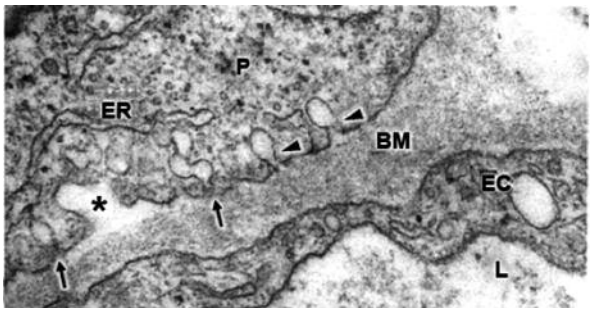

Fig. 9. Brain trauma. Right parieto-temporal subdural haematoma. Transpericytal channels (arrows) communicating the basement membrane (BM) with the pericyte (P) endoplasmic reticulum canaliculi (ER). Some micropinocytotic vesicles exhibit an elongated neck (arrowheads). A swollen basement membrane bifurcation (asterisk) appears extremely dilated. The endothelial cell (EC) and the capillary lumen (L) are also visualized.

cytoplasm with the basement membrane are also found, acting as pathways of facilitated transport. Additionally, uncoated micropinocytotic vesicles, and small and medium sized coated vesicles can be observed. Presumably, the actin and myosin-like filaments speed up the Brownian motion of micropinocytotic vesicles throughout the pericytal cytoplasm.

Pericyte involvement in oedema resolution

Open clathrin-coated and uncoated vesicles are observed connected with the pericyte plasma membrane, and surrounding the Golgi complex area, suggesting a bidirectional macromolecular transport between the basement membrane and the pericyte Golgi compartments: presumably, in one sense, from the basement membrane to the Golgi region, to conduct proteinaceous oedema fluid to be hydrolyzed by Golgi vesicles, as a pericytal mechanism of oedema resolution; and conversely, from the Golgi complex to the plasma membrane, as occurs in normal conditions, to provide plasma membrane and glycocalyx structural constituents [10]. Some micropinocytotic vesicles are also observed orientated to multivesicular bodies, presumably transporting proteins to be degraded by hydrolytic enzymes, as a pericytal mechanism of oedema resolution.

However, in some oedematous areas displaying large extracellular spaces and degenerated myelinated axons, the pericytes exhibit an apparently normal morphology, revealing that certain pericytes maintain their barrier function, and are not activated either by the perifocal brain oedema or by the brain injury [10]. Their submicroscopic features closely resemble the pericytal microglia described by Mori and Leblond [51], the pericytes 'in repose' found by Baron and Gallego [4] in cat cerebral cortex, and the normal pericytes reported by Dodson *et al.* [13].

As earlier postulated by Van Deurs [71] for endothelial cells, it is probable that the following events also occur in pericytes in relationship with its oedema resolution role: a) formation of multivesicular bodies and secondary lysosomes by fusion of micropinocytotic vesicles with small hydrolytic enzymes containing Golgi vesicles, resulting in the formation of pleomorphic dense bodies. This may grow larger by receiving more material to be digested from micropinocytotic vesicles and protein containing vacuoles; b) micropinocytotic vesicles and protein-containing vacuoles might fuse with each other, forming large heterophagosomes or endocytic vacuoles which may appear as multivesicular bodies; c) vacuoles may eventually receive acid hydrolases from small primary lysosomes and develop into large dense bodies; d) secondary lysosomes may remain as residual bodies as observed in complicated brain traumatic lesions, as subdural or extradural haematoma or hygroma [10].

Phagocytic pericytes

In those areas where the blood-brain barrier was severely injured and extravasated erythrocytes were found in the pericapillary space, the pericyte cells revealed phagocytic properties, ingesting whole erythrocytes. Phagocytic pericytes exhibit vacuoles, phagosomes, coated and uncoated micropinocytotic vesicles and lysosomes (Fig. 10).

Pericytal phagocytes have been earlier described in normal and pathological conditions [5,6,8,14,19, 45,46,68,69,71].

Pericytes containing lipofuscin granules and lipid granular deposits

Large lipid droplets and lipofuscin granules appeared accumulated in the pericytal cytoplasm, presumably as a phagocytic response to neighbouring perivascular brain parenchyma destruction or degenerated myelinated axons (Fig. 11).

Dense lipid droplets in pericytes were also reported by Torack [69] at the margin of tumours or in areas of perivascular demyelination. These lipid droplets or secondary lysosomes exhibit a granular coarse osmiophilic material, and are morphologically different from the large dense bodies, apparently primary lysosomes, described by Lafarga and Palacios [34] in pericytes of rat supraoptic nucleus, and by Mato *et al.* [45] in granular pericytes. Semchenko *et al.* [62] described poorly metabolized granules in the pericytal cytoplasm in brain capillaries in brain tumours. Jeynes [31] reported granular pericytes accumulating lipid components in a rabbit cerebrovascular ischaemic model. Tagami *et al.* [66,67] described granular pericytes acting as scavenger cells in stroke-prone spontaneously hypertensive rats. According to Perimutter [54], pericytes are implicated in cerebrovascular amyloid deposition. Presumably some degenerated pericyte populations in traumatic

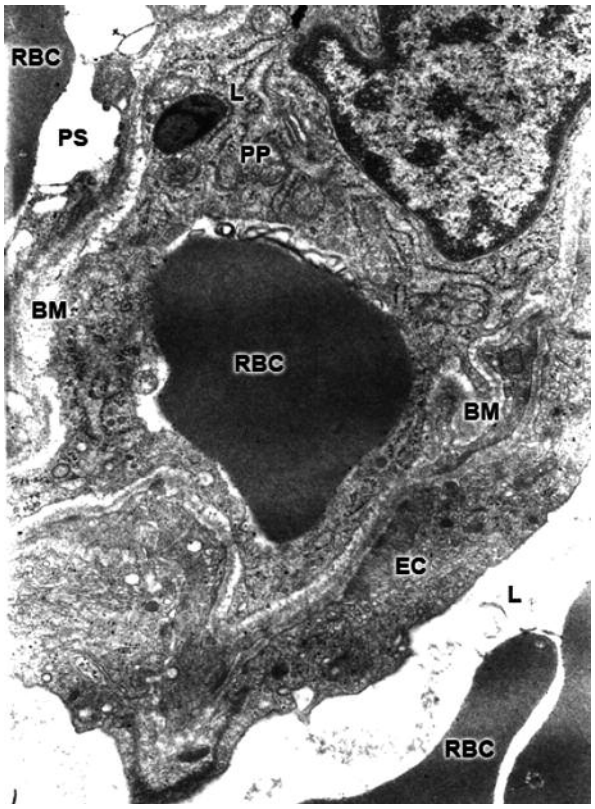

Fig. 10. Brain trauma. Right epidural haematoma. Right parietal cortex. Phagocytic pericyte (PP) embedded within the swollen basement membrane (BM) engulfing a red blood cell (RBC). The cytoplasm shows lysosomes (L) and micropinocytic vesicles (short arrows). The endothelial cell (EC), the capillary lumen (L), and extravasated red blood cells (RBC) are also distinguished.

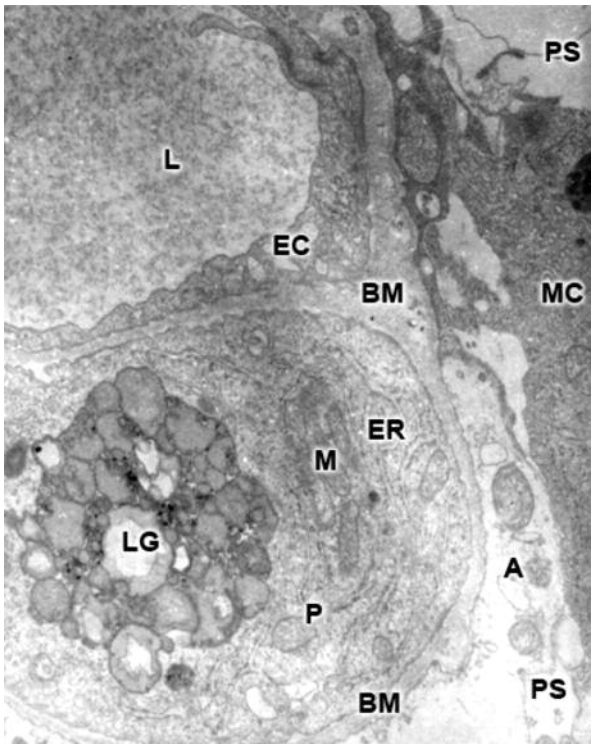

Fig. 11. Brain trauma. Subdural haematoma. Left parietal cortex. Pericytal cytoplasm (P) embedded into the capillary basement membrane (BM) showing a deposit of vacuolated lipid droplets and lipofuscin granules (LG). The capillary lumen (L), the enlarged perivascular space (PS), and a perivascular microglial cell (MC) are also seen.

brain injuries have increased production of amyloid precursor protein, as observed in degenerated pericytes in human brain cultures [72,73]. According to Lupo *et al.* [42], amyloid beta peptides may modulate phospholipid turnover in microvessel pericytes.

Conclusions

In human traumatic brain oedema, pericytes exhibit moderate and remarkable oedematous changes, increased vacuolar and vesicular transport, transient transpericytal channels, and tubular structures demonstrating pericyte brain barrier dysfunction. They show nuclear invaginations, actin and myosin-like filaments, and coupled interaction with endothelial cells through macula occludens revealing their contractile properties. The cytoskeleton is also involved in pericyte contraction and enhanced micropinocytotic transport. Human brain trauma induces pericyte hypertrophic and necrotic changes, and phagocytic capacity. Hypertrophic pericytes induce basement membrane splitting. Degenerated pericytes exhibit lacunar enlargement of the endoplasmic reticulum, dense osmiophilic bodies, glycogen granules, vacuolization, oedematous Golgi apparatus, and pleomorphic mitochondria. Open clathrin-coated and uncoated vesicles are observed connected with the pericyte plasma membrane, and surrounding the Golgi complex area, suggesting a bidirectional macromolecular transport between the basement membrane and the pericyte Golgi compartment, and that pericytes contribute to oedema resolution.

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References

1. Addison DJ, Garner A, Ashton N. Degeneration of intramural pericytes in diabetic retinopathy. Br Med J 1970; 31: 264-266.
2. Allt G, Lawrenson JG. Pericytes: cell biology and pathology. Cell Tissues Organs 2001; 169: 1-11.
3. Ammoury A, Michaud S, Paul C, Prost-Squarcioni C, Alvarez F, Lamant L, Launay F, Bazex J, Chouini-Lalanne N, Marguery MC. Photodistribution of blue-gray hyperpigmentation after amiodarone treatment: molecular characterization of amiodarone in the skin. Arch Dermatol 2008; 144: 92-96.
4. Baron M, Gallego A. The relationship of the microglia with the pericytes in the cat cerebral cortex. Z Zellforsch 1972; 128: 42-57.
5. Bertossi M, Virgintino D, Majorano E, Occhiogrosso M, Roncali L. Ultrastructural and morphometric investigation of human brain capillaries in normal and peritumoral tissues. Ultrastruct Pathol 1997; 21: 41-49.
6. Brierley JB, Brown AW. The origin of lipid phagocytes in the central nervous system: II. The adventitia of blood vessels. J Comp Neurol 1982; 211: 407-417.
7. Cammermeyer J. Juxtavascular karyokinesis and microglia cell proliferation during retrograde reaction in the mouse facial nucleus. Ergeb Anat Entwuiklungsgesch 1965; 38: 1-22.
8. Cancilla PA, Baker RN, Pollock PS, Frommes SP. The reaction of pericytes of the central nervous system to exogenous protein. Lab Invest 1972; 26: 376-383.
9. Castejón OJ. Electron microscopic study of capillary wall in human cerebral edema. J Neuropathol Exp Neurol 1980; 39: 296-328.
10. Castejón OJ. Submicroscopic changes of cortical capillary pericytes in human perifocal brain edema. J Submicrosc Cytol 1984; 16: 601-618.
11. De Gracia DJ, Kreipke CW, Kayali FM, Rafols JA. Brain endothelial HSP-70 stress response coincides with endothelial and pericyte death after brain trauma. Neurol Res 2007; 29: 356-361.
12. Desaki J, Nishida N. A further observation of the structural changes of microvessels in the extensor digitorum longus muscle of the aged rat. J Electron Microsc (Tokyo) 2007; 56: 249-255.
13. Dodson RE, Aoyagi M, Chu LW-F. Ultrastructural changes in subacute cerebral infarction following middle cerebral artery occlusion in the baboon. Cytobios 1975; 13: 97-108.
14. Dodson RE, Tagashira Y, Chu LW-F. Acute pericyte response to cerebral ischemia. J Neurol Sci 1976; 29: 9-16.
15. Dore-Duffy P, Owen C, Balabanov R, Murphy S, Beaumont T, Rafols JA. Pericyte migration from the vascular wall in response to traumatic brain injury. Microvasc Res 2000; 60: 55-69.
16. Dore-Duffy P, Wang X, Mehedi A, Kreripke CW, Rafols JA. Differential expression of capillary VEGF isoforms following traumatic brain injury. Neurol Res 2007; 29: 395-403.
17. Farquhar MG, Hartmann JF. Electron microscopy of cerebral capillaries. J Neuropathol Exp Neurol 1956; 15: 18-39.
18. Fisher M, French S, Ji P, Kim RC. Cerebral microbleeds in the elderly: a pathological analysis. Stroke 2010; 41: 2782-2875.
19. Frey A, Meckelein B, Weiler-Guttler H, Mockel B, Flach R, Gassen HG. Pericytes of the brain microvasculature express gamma-glutamyl transpeptidase. Eur J Biochem 1991; 202: 421-429.
20. Frontczak-Baniewicz J, Olszewska H, Gadamski R, Barskow I, Gajkowska B. Alterations in rat's brain capillaries in a model of focal cerebral necrosis. Exp Toxicol Pathol 2000; 52: 77-85.
21. Glees P, Hasan M, Voth D, Schwarz M. Fine structural features of the cerebral microvaculature in hydrocephalic human infants: correlated clinical observations. Neurosurg Rev 1989; 12: 315-321.
22. Hayden MR, Karuparthi PR, Habibi J, Wasekar C, Lastra G, Manrique C, Stas S, Sowers JR. Ultrastructural islet study of early fibrosis in the Ren2 rat model of hypertension. Emerging role of the islet pancreatic pericyte-stellate cell. JOP 2009; 8: 725-738.
23. Hayden MR, Karuparthi PR, Habibi J, Lastra G, Patel K, Wasekar C, Manrique CM, Ozerdem U, Stas S, Sowers JR. Ultrastructure of islet microcirculation, pericytes and the islet exocrine interface in the HIP rat model of diabetes. Exp Biol Med 2008; 233: 1109-1123.
24. Hayden MR, Patel K, Habibi J, Gupta D, Tekwani SS, Whaley-Connell A, Sowers JR. Attenuation of endocrine-exocrine pancreatic communication in type 2 diabetes: pancreatic extracellular matrix ultrastructural abnormalities. J Cardiometab Syndr 2008; 3: 234-243.
25. Gerrits PO, de Weerd H, van der Want JJ, Kortekaas R, Luiten PG, Veening JG. Microvascular changes in estrogen-α sensitive brainstem structures of aging female hamsters. Neurosci Res 2010; 67: 267-274.
26. Gonul E, Duz B, Kahraman S, Kayali H, Kubar A, Tumurkaynak E. Early pericyte response to brain hypoxia in cats: an ultrastructural study. Microvasc Res 2002; 64: 116-119.
27. Hayashi K, Nakao S, Nakaoke R, Nakagawa S, Kitagawa N, Niwa M. Effects of hypoxia on endotelial/pericytic co-culture modelo f the blood-brain barrier. Regul Pept 2004; 123: 77-83.
28. Herman IJ, Jacobson S. In situ analysis of microvasculr pericytes in hypertensive rat brain. Tissue Cell 1988; 20: 1-12.
29. Herman IM, Newcomb PM, Coughlin JE, Jacobson S. Characterization of microsvascular cell cultures from normotensive and hypertensive rat brains: pericyte-endothelial cell interactions in vitro. Tissue Cell 1987; 19: 197-206.
30. Hills CP. Ultrastructural changes in the capillary bed of the rat cerebral cortex in anoxic-ischemic brain lesions. Am J Pathol 1964; 44: 531-544.
31. Jeynes B. Reactions of granular pericytes in a rabbit cerebrovascular ischemia model. Stroke 1985; 16: 121-125.
32. Kitamura T. The origin of brain macrophages. Some cosiderations on the microglia theory of Del Rio Hortega. Acta Pathol Jpn 1973; 23: 11-26.

33. Kosunen TU, Waksman BH, Samuelsson IK. Radioautographic study of cellular mechanisms in delayed hypersensitivity. J Neuropathol Exp Neurol 1963; 22: 367-380.
34. Lafarga M, Palacios G. Ultrastructural study of pericytes in the rat supraoptic nucleus. J Anat 1975; 120: 433-438.
35. Le Beux YJ, Willemot J. Actin-like filaments in the endothelial cells of adult rat brain capillaries. Exp Neurol 1978a; 58: 446-454.
36. Le Beux YJ, Willemot J. Actin and myosin-like filaments in rat brain pericytes. Anat Rec 1978b; 190: 811-826.
37. Lewandowska E, Szpak GM, Wierzba-Bobrowicz T, Modzelewska J, Stępień T, Pasennik E, Schmidt-Sidor B, Rafałowska J. Capillary vessel wall in CADASIL angiopathy. Folia Neuropathol 2010; 48: 104-115.
38. Li C, Zhang WF, Zhao YF. Pericytes may have important role in the pathogenesis of vascular malformation. Med Hypothesis 2007; 68: 808-810.
39. Li YJ, Wen G, Yang L, Zhang XL. Heterogeneity of angioarchitecture and their hemodynamic changes in benign and malignant breast tumors. Zhonghua Zhong Liu Za Zhi 2009; 31: 24-27.
40. Liwnicz BH, Leach JL, Yeh HS, Privitera M. Pericyte degeneration and thickening of basement membranes of cerebral microvessels in complex partial seizures: electron microscopic study of surgically removed tissue. Neurosurgery 1990; 26: 409-420.
41. Liu HM. Neovascullature and blood-brain barrier in ischemic brain infarct. Acta Neuropathol 1988; 75: 422-426.
42. Lupo G, Anfuso CD, Assero G, Strosznajder RP, Walski M, Pluta R, Alberghina M. Amyloid beta (1-42) and its beta (25-35) fragment induce in vitro phosphatidylcholine hydrolysis in bovine retina capillary pericytes. Neurosci Lett 2001; 303: 185-188.
43. Majno G, Shea SM, Leventhal M. Endothelial contraction induced by histamine type mediators. J Cell Biol 1969; 42: 647-672.
44. Markov DV, Dimova RN. Ultrastructural alteration of rat brain microglia cells and pericytes after chronic lead poisoning. Acta Neuropathol (Berlin) 1974; 28: 25-35.
45. Mato M, Ookawara S, Kurihora K. Uptake of exogenous substances and marked infoldings of the fluorescent granular pericytes in cerebral fine vessels. Am J Anat 1980; 157: 329-332.
46. Maxwell DS, Kruger L. Small blood vessels and the origin of phagocytes in the rat cerebral cortex following heavy particle irradiation. Exp Neurol 1965; 12: 33-54.
47. Maynard EA, Schultz RL, Peace DC. Electron microscopy of the vascular bed of the rat cerebral cortex. Am J Anat 1957; 100: 409-433.
48. Mc Donald LW, Hayes L. The role of capillaries in the pathogenesis of delayed radionecrosis of brain. Am J Pathol 1967; 50: 745-764.
49. Medrado A, Costa T, Prado T, Reis S, Andrade Z. Phenotype characterization of pericytes during tissue repair following low-level laser therapy. Photodermatol Photoimmunol Photomed 2010; 2: 192-197.
50. Melgar MA, Rafols J, Gloss D, Diaz FG. Postischemic reperfusion: ultrastructural blood-brain barrier and hemodynamic correlative changes in an awake model of transient forebrain ischemia. Neurosurgery 2005; 56: 571-581.
51. Mori S, Leblond CP. Identification of microglia in light and electron microscopy. J Comp Neurol 1969; 135: 57-80.
52. Owman Ch, Edvinsson L, Hardebo JE, Croshel-Stewari U, Unsicker K, Walles B. Immunohistochemical demonstration of actin and myosin in brain capillaries. In: Cervós-Navarro J, Betz E, Ebhardt G, Ferst R, Wullemweber R (eds.). Advances in Neurology. Vol. 20. Raven Press, New York 1978; pp. 347-352.
53. Pavlov KA, Chekmaryova IA, Shchyogolev AI, Mishnyov OD. Ultrastructural characteristics of peripheral arteriovenous and venous angiodysplasias. Bull Exp Biol Med 2009; 147: 480-484.
54. Perimutter LS. Microvascular pathology and vascular basement membrane components in Alzheimer's disease. Mol Neurobiol 1994; 9: 33-40.
55. Persson L. Cellular reactions to small cerebral stab wounds in the rat frontal lobe. An ultrastructural study. Virchows Arch B Cell Pathol 1976; 18: 21-37.
56. Piquer-Gil M, García-Verdugo JM, Zipancic I, Sánchez MJ, Alvarez-Dolado M. Cell fusion contributes to pericyte formation after stroke. J Cereb Blood Flow Metab 2009; 29: 480-485.
57. Popova EN, Zagrebina OV. Ultrastructure of the blood-brain barrier in the cerebral cortex in atherosclerotic dementia. Morfologiia 1998; 114: 25-30.
58. Rensink AA, Otte-Holler I, De Boer R, Bosch RR, Ten Donkelaar HJ, De Waal RM, Verbeek MM, Kremer B. Insulin inhibits amyloid beta-induced cell death in cultured human brain pericytes. Neurobiol Aging 2004; 25: 93-103.
59. Robinson JC, Challa VR, Jones DS, Kelly DL Jr. Pericytosis and edema generation: a unique clinicopathologica variant of meningioma. Neurosurgery 1996; 39: 700-706.
60. Rouget C. Sur la contractilite des capillaires sanguins. C R Acad Sci (Paris) 1879; 88: 916.
61. Schlingemann RO, Rietveld FJ, de Waal RM, Ferrone S, Ruiter DJ. Expression of the high molecular weight melanoma-associated antigen by pericytes during angiogenesis in tumors and in healing wounds. Am J Pathol 1990; 136: 1393-1405.
62. Semchenko VV, Stepanov SS, Savchenko A. Ultrastructural manifestation of brain edema-swelling in neurooncologic patients. Zh Vopr Neirokhir Im N N Burdenko 1984; 1: 16-20.
63. Shi X. Cochlear pericyte responses to acoustic trauma and the involvement of hypoxia-inducible factor-1alpha and vascular endothelial growth factor. Am J Pathol 2009; 174: 1692-1704.
64. Szpak GM, Lewandowska E, Wierzba-Bobrowicz T, Bertrand E, Pasennik E, Mendel T, Stępień T, Leszczyńska A, Rafałowska J. Small cerebral vessel disease in familial amyloid and non-amyloid angiopathies: FAD-PS-1 (P117L) mutation and CADASIL. Immunohistochemical and ultrastructural studies. Folia Neuropathol 2007; 45: 192-204.
65. Stensaas LJ. Pericytes and perivascular microglial cells in the basal forebrain of the neonatal rabbit. Cell Tissue Res 1975; 158: 517-541.
66. Tagami M, Nara Y, Kubota A, Fujino H, Yamori Y. Ultrastructural changes in cerebral pericytes and astrocytes of stroke-prone spontaneously hypertensive rats. Stroke 1990; 21: 1064-1071.
67. Tagami M, Kubota A, Nara Y, Yamori Y. Detailed disease processes of cerebral pericytes and astrocytes in stroke-prone SHR rats. Clin Exp Hypertens A 1991; 13: 1069-1075.
68. Thomas WE. Brain macrophages: on the role of pericytes and perivascular cells. Brain Res Brain Res Rev 1999; 31: 41-57.

69. Torack RM. Ultrastructure of capillary reaction to brain tumors. Arch Neurol 1961; 5: 86-98.
70. Van der Avoort IA, van der Laak JA, Otte-Höller I, van de Nieuwenhof HP, Massuger LF, de Hullu JA, van Kempen LC. The prognostic value of blood and lymph vessel parameters in lichen sclerosus for vulvar squamous cell carcinoma development: an immunohistochemical and electron microscopy study. Am J Obstet Gynecol 2010; 203: 167-168.
71. Van Deurs B. Observations on the blood-brain barrier in hypertensive with particular references to phagocytic pericytes. J Ultrastruct Res 1976; 56: 65-77.
72. Verbeek MM, De Waal RM, Schipper JJ, Van Nostrand WE. Rapid degeneration of cultured humanbrain pericytes by amyloid beta protein. J Neurochem 1997; 68: 1135-1141.
73. Verbeek MM, Van Nostrand WE, Otte-Holler I, Wesseling P, De Waaj RM. Amyloid-beta-induced degeneration of human brain pericytes dependent on the apolipoprotein E genotype. Ann NY Acad Sci 2000; 903: 187-199.
74. Wegiel J, Wisniewski HM. Tubuloreticular structures in microglial cells, pericytes and endothelial cells in Alzheimer's disease. Acta Neuropathol 1992; 83: 653-658.
75. Yamagishi S, Imaizumi T. Pericyte biology and diseases. Int J Tissue React 2005; 2: 125-135.