

Long-term consequences of surgical brain injury – characteristics of the neurovascular unit and formation and demise of the glial scar in a rat model

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Abstract

Neurosurgical procedures often involve, as a result of the surgeon approaching the diseased region, unavoidable trauma to the adjacent, non-diseased brain structures. Because of the priority of removing the immediate threats to health or life that underlie such interventions, side effects of surgical brain injuries were not given much attention until recently. The cause-and-effect association of the eventual delayed cognitive and/or neurological deficits is often obscure due to substantial potential of the brain for compensatory changes, long life span in man, and aging-related phenomena. However, animal and human studies have demonstrated that physical insults to the brain can initiate a cascade of changes that results, in the long run, in massive neurodegeneration and brain atrophy. Here we present a review of morphological and ultrastructural findings obtained mostly in a rat model of surgical neocortex injury, with consideration of the current view of this region as a network of specific neurovascular units. The neurovascular unit is a dynamic assembly consisting of a capillary vessel, pericytes, neurons and capillary-bound astrocytes. The integrity of this building block and the interactions between its component parts are responsible, among other things, for the proper functioning of the blood-brain barrier, brain blood circulation and the brain as a whole, and its dysregulation is associated with many CNS pathologies. The studies performed in the rat model of surgical brain injury presented in this review have brought new interesting findings regarding the consequences of such damage to the (ultra)structure and hence presumably to the function of the unit. These findings may have some relevance to human clinical situations.

Key words: angiogenesis, brain injury, delayed cell death, glial scar, morphology, neurosurgery, neurovascular unit, ultrastructure.

Introduction

Life-saving neurosurgical procedures, e.g. removal of a brain tumour or clipping of an aneurysm, often in-

volve unavoidable trauma to neighbouring, non-diseased brain tissues. For instance, this is the case in transcortical approaches that involve an incision or removal of a piece of non-diseased neocortex and sta-

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unching the related haemorrhage that may give rise to additional brain damage.

Delayed consequences of surgical brain injury (SBI) have not been given much attention until very recently. It was only a few years ago that a rat model of SBI was first employed for studying the respective phenomena at cellular and subcellular levels and relating them to the underlying biochemical changes [32]. In this model, the SBI is made by excising, under ketamine/xylazine anaesthesia, a moderate-sized piece of sensorimotor cortex in the frontotemporal brain region in male Wistar rats (Fig. 1A). This model, which has been used throughout our studies included in this review, imitates well the respective human neurosurgery situation in that it involves the most typical early consequences of SBI, such as brain oedema and neuronal death. A similar model has been introduced and explored by others [46,47,53]. However, those studies focused mostly on investigating

the effects of a variety of experimental drugs meant to prevent or minimize early consequences of such trauma, and not on long-term consequences of the surgery itself. This review was aimed at characterization of certain phenomena taking place in the cerebral cortex after SBI, with particular consideration of cell ultrastructure and long-term morphological consequences of this procedure.

Structure and function of the neurovascular unit

Efficient functioning of both the central nervous system (CNS) and the blood-brain barrier (BBB) depends on proper functioning of the neurovascular unit (NVU) – a dynamic structure made of neurons, capillary vessel (consisting of endothelial cells, pericytes and basement membrane), interstitial extracellular matrix and vessel-bound astrocytes [1,11,61].

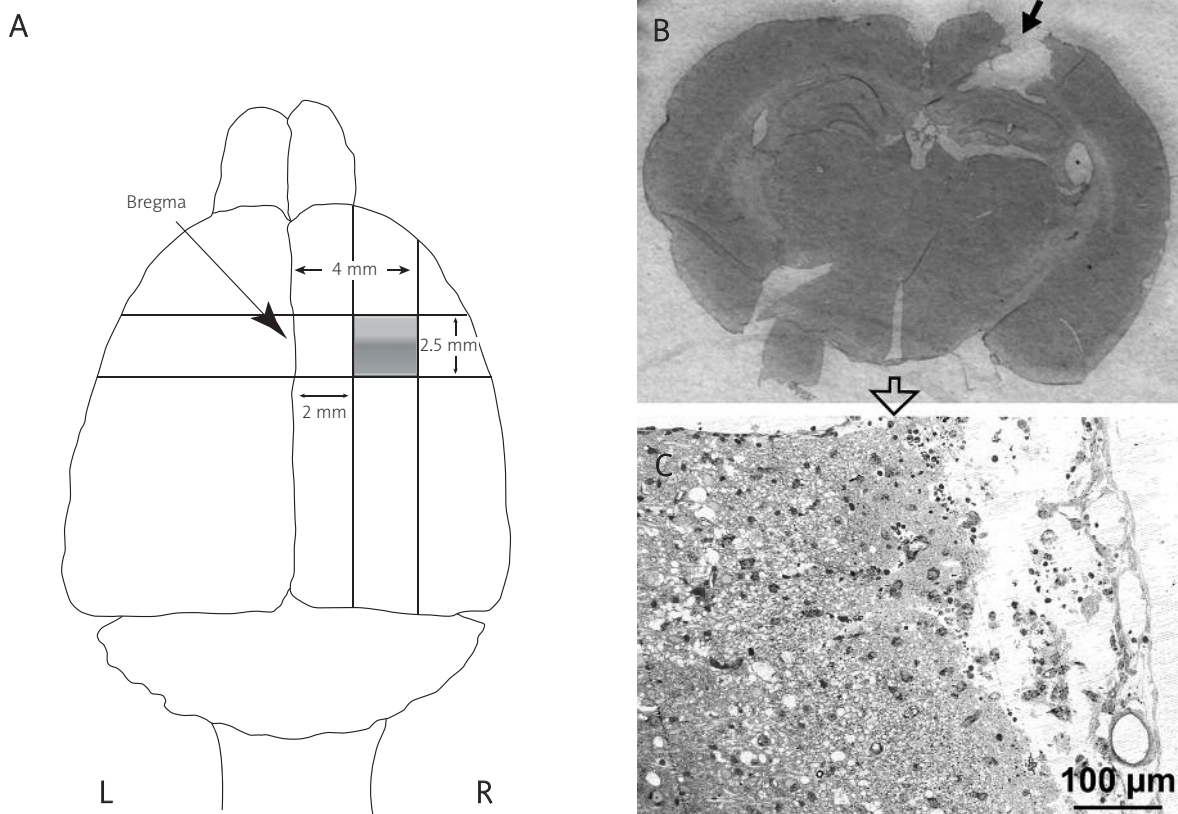


Fig. 1. Panel A: Location of the surgical brain injury in the studied model. The depth of the lesion was about 2 mm. Panel B: Cortical tissue loss on post-SBI day 4 (coronal cross-section at Bregma 2.8 mm). Note the expansion of brain damage (arrow) far beyond the most caudal aspect (\approx Bregma 1.3 mm) of the original SBI. Panel C: Light microscopy (toluidine blue staining) picture of a semi-thin (1 μ m) slice from peri-lesion area showing cortical oedema evidenced by spongy parenchyma.

NVU dysregulation is associated with many CNS pathologies [79]. The concept of the NVU as the basic unit that is responsible for normal brain functioning assumes a complex network of interactions between all its component parts [51].

Capillary vessels

Capillary vessels in the CNS are normally built of a single layer of endothelial cells encompassed abuminally by the basement membrane that is penetrated by astrocytic and occasional endothelial processes. The endothelial cells are connected with each other by desmosomal tight junctions that are impermeable to high molecular weight compounds and greatly limit the passage of low molecular weight substances [38,51]. A characteristic feature of brain capillary endothelium is the absence of fenestration.

Pericytes

Pericytes are a relatively small subset of vessel-bound cells (in the rat brain the average ratio of these cells to endothelial cells is about 1 : 5). They populate the basement membrane that encompasses capillary endothelium and maintain a tight structural relationship with endothelial cells via both peg-and-socket contacts and gap junctions [21]. The presence of contractile proteins in their cytoplasm indicates that they play a role in the regulation of capillary blood flow. It is also known that these cells can migrate from the capillaries to brain parenchyma in hypoxia and after brain trauma, i.e. under the conditions that cause BBB permeabilization [7,21,34]. It is not yet clear if the migration plays a considerable role in the BBB malfunctioning. Apart from producing components of the basement membrane and playing an important regulatory role due to their production of many growth/proangiogenic factors [22], pericytes are a source of adult multipotent stem cells that can differentiate into smooth muscle cells, fibroblasts, and macrophages [21].

Astrocytes

Astrocytic processes cover almost 9/10 of the abluminal side of the basement membrane of brain capillaries [69]. Therefore, it has been postulated that these cells co-determine the integrity of BBB [18]. Later studies have shown that astrocytes also affect the morphogenesis and organization of brain capillary walls, that the glial cell-derived neurotro-

phic factor (GDNF) released by these cells plays an important role in BBB maturation [2,37,77], and that they also contribute to the regulation of BBB permeability [6,9,89].

Neurons

According to the concept of NVU, neurons are an integral part of this makeup [51]. Because of their role in the reception, transduction and analysis of the stimuli arriving in the CNS, and in the initiation and execution of bodily reactions to the stimuli, neurons are usually the main target in studies on CNS pathologies. However, they are in permanent contact with the other components of the NVU, and both the condition and behaviour of all these elements are mutually modulated [41]. Therefore, the neuronal degeneration caused by brain trauma should be considered in the context of neurons' relationship to the other NVU constituents.

Collapse and reconstruction of the neurovascular unit following surgical brain injury

Disturbances consequential to SBI do not concern brain blood flow or neurons alone – in this case the entire CNS homeostasis is affected. Neurodegeneration and angiogenesis begin within a few days after the injury, i.e. during the period of extensive expansion of the primary damage (see Fig. 1B). SBI also causes disruption of signal transduction and perturbation of the interactions between NVU elements. Reaction(s) of these elements do not end at an early post-injury phase, but persist – with some modifications – during the consequential remodeling and 'repair' of the injured cortex.

Most brain insults are linked to BBB permeabilization or opening. This is supported, among other findings, by ultrastructural studies [52,57-59]. At an early phase of BBB impairment there is an increased fluid flow into the brain parenchyma, which ultrastructurally manifests with enhanced pinocytosis in the otherwise unchanged capillary endothelium. This condition, if persistent, results in endothelial cell damage and tight junctions' failure. During the intense fluid influx the brain parenchyma is penetrated by albumins, fibronectin and fibrinogen [60], followed by the commencement of angiogenesis. The brain's response to a mechanical injury or infarct also involves a number of inflammatory phenomena,

including activation of microglia and astrocytes, and macrophage infiltration [19,44,90].

In the presented model of SBI, the border zone of the surgically made cavity soon (within a few hours) showed perivascular astrocytic oedema and infiltration of brain parenchyma by leukocytes, monocytes and macrophages. Two days after the injury, massive angiogenesis was already evident in this region [32,81]. This phenomenon, which has also been reported in other models of traumatic brain damage and in brain ischaemia [26,55,65,85], is considered a key mechanism in brain adaptation to hypoxia. This view is based, among other things, on the observation that the density of brain cortex capillaries in rats subject to a continuing generalized hypoxia can markedly increase over just a few weeks and normalize similarly fast after restoring normoxia [63].

The mechanism of post-SBI formation of new vessels differed depending on the distance from the injury site. In close vicinity to the lesion, the new capillaries were formed *de novo* by cells with endothelial characteristics (see below), whereas in more distal regions the new vessels were usually formed via the well-known bridging of the existing capillaries [81].

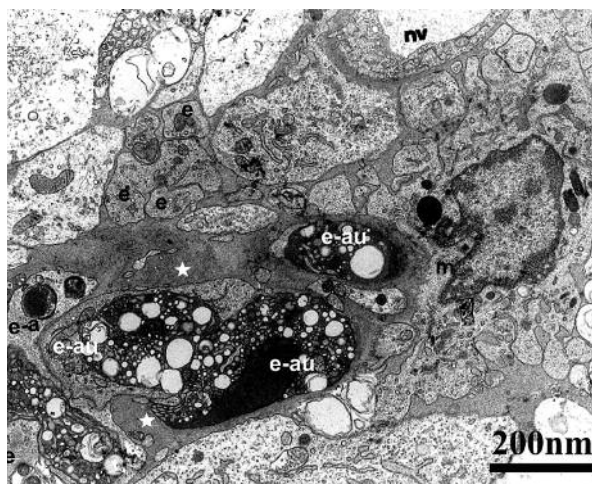


Fig. 2. The area in the vicinity of SBI showing the presence of extravasated plasma proteins (white asterisks) and an aggregate of endothelial cell processes (e), 4 days post-SBI; m – macrophage, e-a – apoptotic endothelial cell with typical condensed nuclear chromatin, e-au – autophagic endothelial cell, nv – new vessel (reprinted with kind permission from Acta Neurobiologiae Experimentalis [1]).

The formation of new capillary vessels is usually preceded by permeabilization of the walls of ‘old’ (i.e. pre-SBI) capillaries and extravasation of serum proteins that make a scaffold for the new vessels [23]. In the presented SBI model, BBB damage should be considered a consequence both of mechanical damage to the vessels and of action of free oxygen radicals that are generated at an increased rate under such circumstances [56]. The area near the cortical wound was found to show extravascular presence of serum proteins (Fig. 2), which was most likely the result of BBB opening in the injury zone and the neighbouring region that showed astrocytic oedema [32]. This is an important finding because of the postulated opposing roles for these proteins: one beneficial, as a matrix for the new capillaries; and the other one detrimental, as an inducer of neuronal and astrocytic cells’ damage [14].

The observations from our model of SBI are in line with the dual role of the extravasated proteins. The region showing the presence of these proteins contained both degenerating and dying neurons, and oedematous astrocytes. This zone also encompassed complexes of atypical endothelial cells carrying specific cytoplasmic fibrils (see ‘‘Characteristics

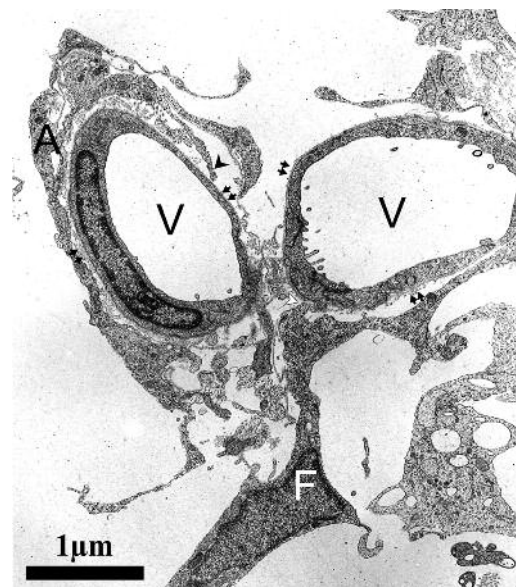


Fig. 3. Astrocyte (A) and fibroblast (F) in the vicinity of new capillary vessels (V), 4 days post-SBI. Astrocytic (black arrowhead) and fibroblast (white arrowhead) processes make direct contact with the capillaries surrounded by a basement membrane-like material (black arrows).

of the endothelial cells...” section below), suggesting the formation of new capillaries [32]. Some of the newly formed (as evidenced by high, hypertrophic endothelium) vessels, which appeared already on post-SBI day 4, had the appearance of complete, ultrastructurally normal, basement membrane-ensheathed capillaries surrounded by astrocytic processes. However, many novel capillaries lacked regular basement membrane and were encased in a basement membrane-like material instead (Fig. 3). That was despite the fact that the adjacent pericytes (if present, see below), astrocytes and neurons, and in most cases also endothelial cells, were of ultrastructurally normal appearance [32]. The latter observation is in obvious contrast to what is usually seen in neurodegeneration-affected brains, e.g. in Alzheimer’s disease patients [27].

Some of the newly formed capillaries found in the zone of SBI and its vicinity, which were identified by hyperplastic endothelium, lacked pericytes in their walls [28]. Such vessels are likely dysfunctional, because normal functioning of brain capillaries requires not only endothelial maturity, but also functional pericyte-endothelial cell connections. Pericytes are known to take part in the regulation of endothelial cells’ proliferation, migration, survival and differentiation, and possibly also contribute to the control of blood flow and vessel wall permeability [12,21]. The observations made regarding pericytes in the presented model are consistent with the established view on the role of these cells in brain blood vessels [21].

Formation of new capillaries was still apparent 30 days post-SBI. Two months later (our studies did not cover the intervening period) both the basement membrane of the new vessels and the perivascular space showed the presence of collagen fibres, the emergence of which is a well-known consequence of BBB failure. The scar border of the posttraumatic cavity was separated from normal brain parenchyma by a basement membrane-like material that filled the space between the new and pre-existing cells.

From post-SBI day 4 on, the peri-lesion area abounded with astrocytes showing the presence of glial fibrillary acidic protein (GFAP) [31]; Fig. 4, top panels. At the same time, the astrocytes in the vicinity of the lesion showed the presence of vimentin (Fig. 4, lower panels), which is considered a characteristic of immature astrocytes, but has also been reported to associate with injury-related astrocytic proliferation [5].

Formation and demise of the glial scar

Beginning on post-SBI day 4, the peri-wound area showed an increase in both the number and hypertrophy of astrocytes. It was not clear whether it was the result of the influx of the cells, or of their proliferation *in situ*, or both. Simultaneously, this area revealed the presence of fibroblast-like cells that likely migrated into the scar region from damaged meninges or were the descendants of these cells. Interestingly, a major subset of the cells was heavily loaded with intracytoplasmic lipid droplets (Fig. 5). The presence of lipofibroblasts in the rodent brain has been reported only recently [28], and their role in this organ has not yet been elucidated. However, fibroblasts and lipofibroblasts were reported to be critically involved in homeostasis and injury/repair in rodent lung [75], which finding raises the possibility that they may play a similar role in the brain after SBI. Notably, lung fibroblasts secrete leptin [75], which modulates adhesion and the homing potential of endothelial progenitor cells (EPC) and may promote angiogenesis [70]. On the other hand, leptin may also cause endothelial dysfunction of lung vessels through a variety of mechanisms [50]; however, it is not clear if the same mechanisms are operative in the brain. The lipofibroblast-like cells were usually found in close proximity to new capillaries that showed an unusual characteristic: their walls were built of more than one layer of endothelial cells (Fig. 5). The luminal surface of the latter was occasionally endowed with multiple microvilli, which are known to promote adherence of morphotic blood elements to vessel walls, suggesting endothelial cell pathology. The microvilli-rich endothelial cells usually also showed an increased electron ‘density’ (see Fig. 5), which is a common finding in cells entering apoptosis [87]. Ultrastructural examination revealed that the apoptotic cells constituted less than 5% of the total endothelial cell population in the newly forming capillaries.

A well-formed glial scar was apparent in the 5th post-SBI week. Its component astrocytes retained a hypertrophic appearance and abounded in GFAP, but showed no ultrastructural abnormalities at that time. However, three months after the SBI their processes began to show ultrastructural symptoms of oedema, and shortly thereafter the scar presented signs of lysis and dissolution. Simultaneously, single and bundled collagen fibrils of either pericytic or fibroblast origin appeared in the scar region, revealing ongoing perivascular fibrosis. The delayed lytic

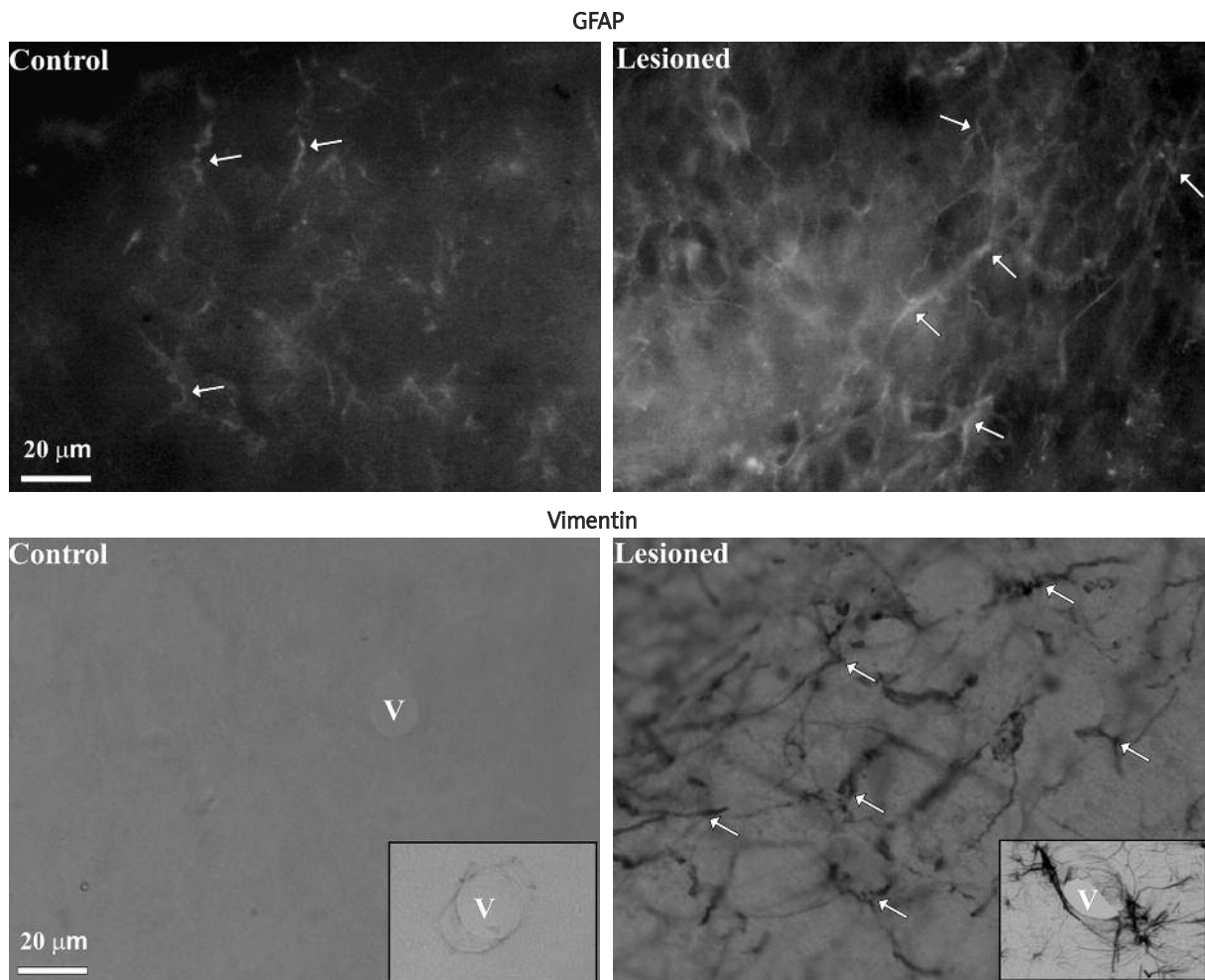


Fig. 4. Upper panels: GFAP immunoreactivity in the cortex from control (sham-operated) and lesioned rats, 4 days post-SBI. Cells from the control rat (left micrograph) show weak GFAP staining (arrows); cells from the lesioned (SBI) rat (right micrograph) show enhanced GFAP expression. Lower panels: Vimentin immunoreactivity in the cortex from control and lesioned rats. No vimentin immunostaining is visible in the control rat (left microphotograph), whereas the cortex from its lesioned counterpart (right micrograph) shows intense vimentin immunostaining (arrows). The inserts show vimentin-positive non-capillary blood vessels (V) from control and lesioned rats. Note that not all the brain vessels were vimentin-positive.

phenomena showed time-related progression and eventually, despite the continuing scarring, resulted in – first ultrastructurally (Fig. 6) and then macroscopically detectable – dissolution of the scar, its neighbouring cortex and the adjacent structures. The expansion of the secondary damage was associated with ongoing scarring-related phenomena including the angiogenesis that retained the aforementioned dependence between the mechanism of new vessel formation and the distance from the zone of decaying parenchyma. This zone showed continuing presence of macrophages and fibroblasts that likely were of

meningeal or non-CNS origin (see above), but might also be the descendants of CNS pericytes that have stem cell potential [21]. These findings clearly demonstrated instability of the primary scar despite the apparent completion of its formation three months post-SBI [31]. Twelve months after the SBI, the loss of brain parenchyma often reached the 3rd ventricle (see Fig. 7). Despite the extensive loss of tissue involving both the cortex and subcortical structures (e.g. the striatum) of the injured hemisphere, which may result from both retrograde and transneuronal degeneration, the rats showed no obvious behavioural aberrations.

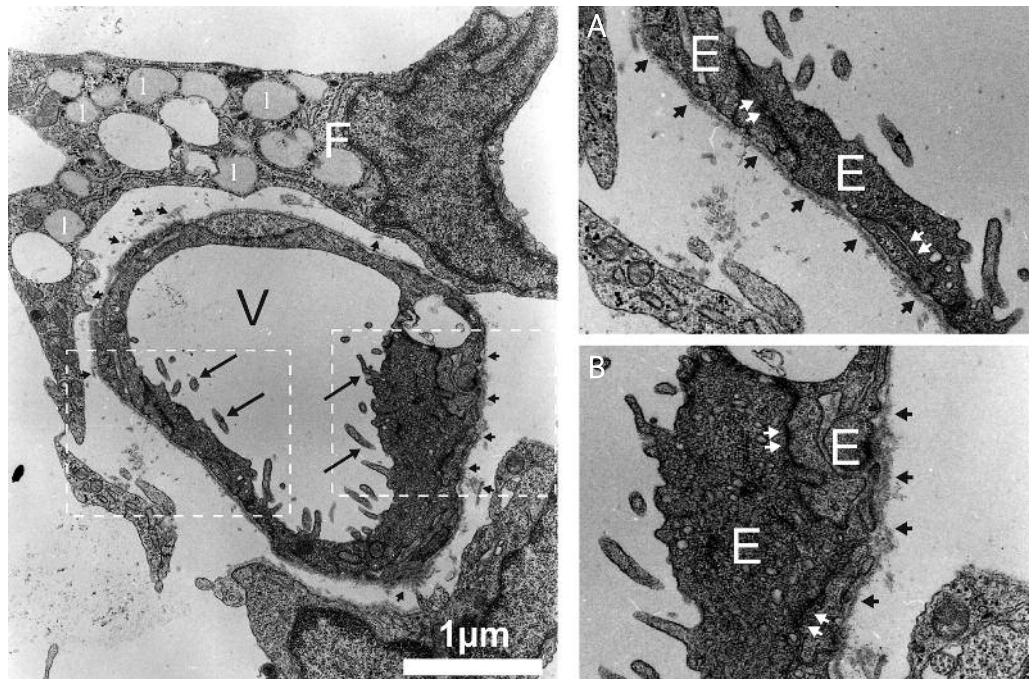


Fig. 5. Left side micrograph showing a newly formed capillary (V) and a neighbouring fibroblast-like cell (F) carrying multiple lipid droplets (l), 4 days post-SBI. The vessel is surrounded by a basement membrane-like material (short black arrows), and its wall is formed by a double layer of endothelial cells (for expanded view of the dashed white line-delimited portions of the image see panels “a” and “b”); twin white arrows in the right panels show the border between the contacting endothelial cells (E). The luminal surface of the endothelial cell in the lower right quadrant of the vessel forms multiple processes (long black arrows). Note the enhanced electron density of the cell compared to that of its neighbours.

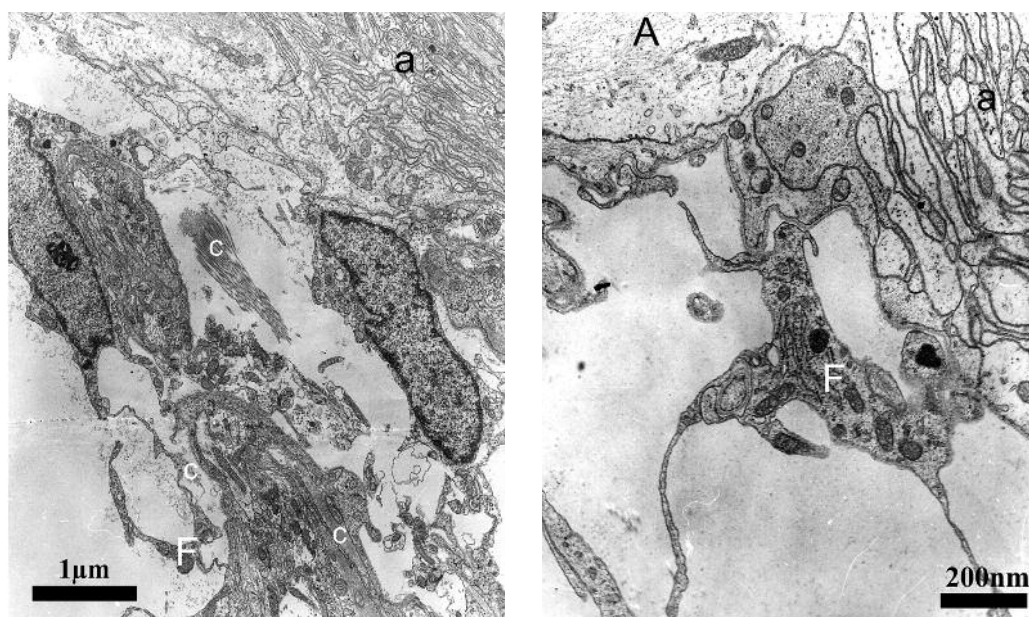


Fig. 6. Peri-lesion region, 6 months post-SBI. Left panel shows a collagen (c) producing fibroblast-like cell (F) and swollen processes (a) of a presumably necrotic astrocyte at the scar site. Right panel shows a necrotic astrocyte (A) and ‘empty’ astrocytic processes (a).

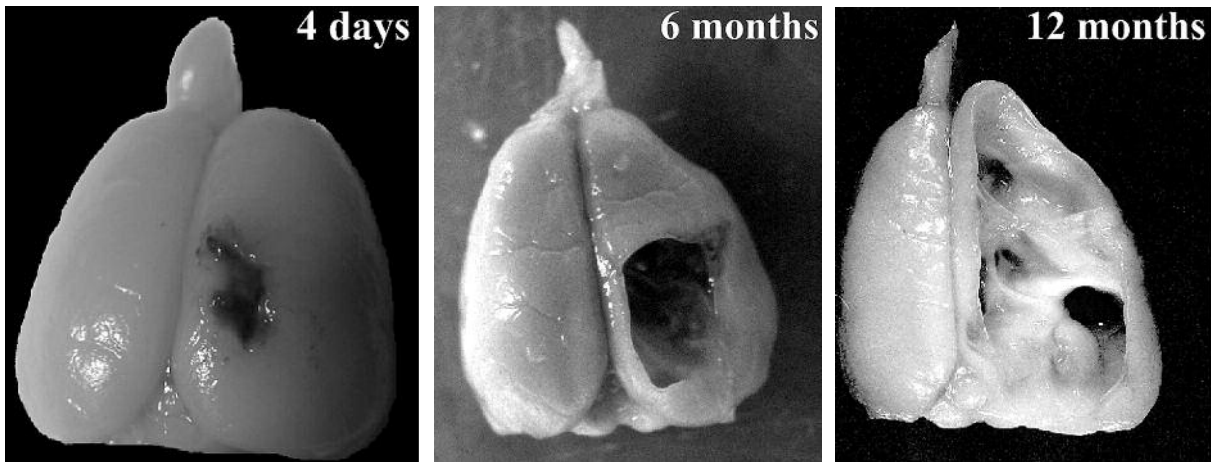


Fig. 7. Macroscopic view of representative rat brains at different times post-SBI. Note the time-dependent expansion of tissue loss.

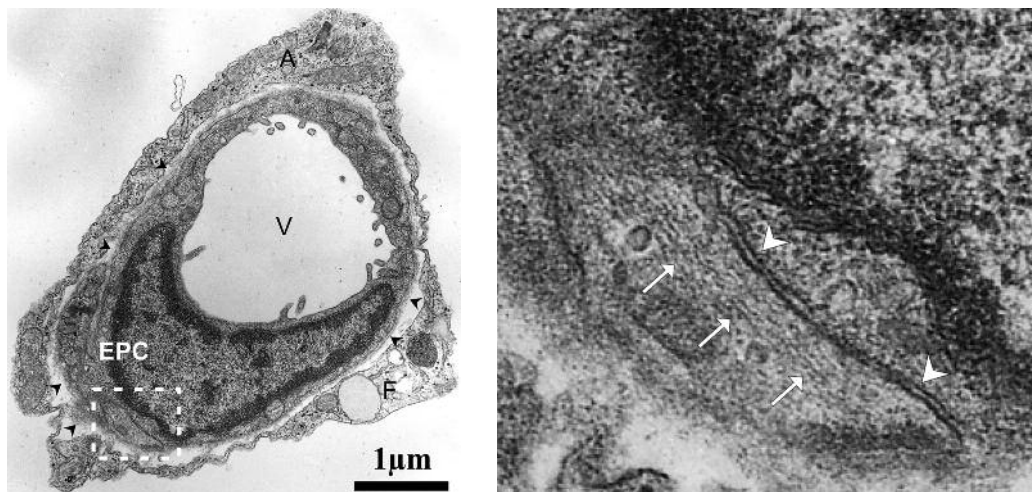


Fig. 8. Micrograph showing a newly formed capillary vessel (V) in the lesion region, 4 days post-SBI. EPC – presumed endothelial progenitor cell, A – astrocyte, F – fibroblast-like cell process showing the presence of prominent rough endoplasmic reticulum and a large lipid droplet; black arrowheads point at basement membrane-like material. Expanded view of the dashed line-delimited part of the micrograph (right panel) shows the presence of characteristic fibrils of ≈ 8 nm diameter (arrows) in the vicinity of the cell membrane (white arrowheads). Arrowheads show the place of contact with the neighbouring EPC.

tions. However, their actual neurological status is yet to be established by more refined measures.

Characteristics of the endothelial cells forming new vessels

Brain trauma induces the emergence of EPC in the brain [33,55,67]. The SBI-induced angiogenesis resulted in formation of new capillaries of mostly

typical morphology [28]. However, some of the capillary wall-forming endothelial cells showed in their cytoplasm the presence of unusual cytoplasmic fibrils of 5-8 nm diameter (Fig. 8). This finding made us suppose that they represented EPC, and became the impulse for their better characterization. Groups of these cells were already apparent on post-SBI day 2 amidst extravasated plasma proteins that included the easily recognizable fibrin fibres; this suggested

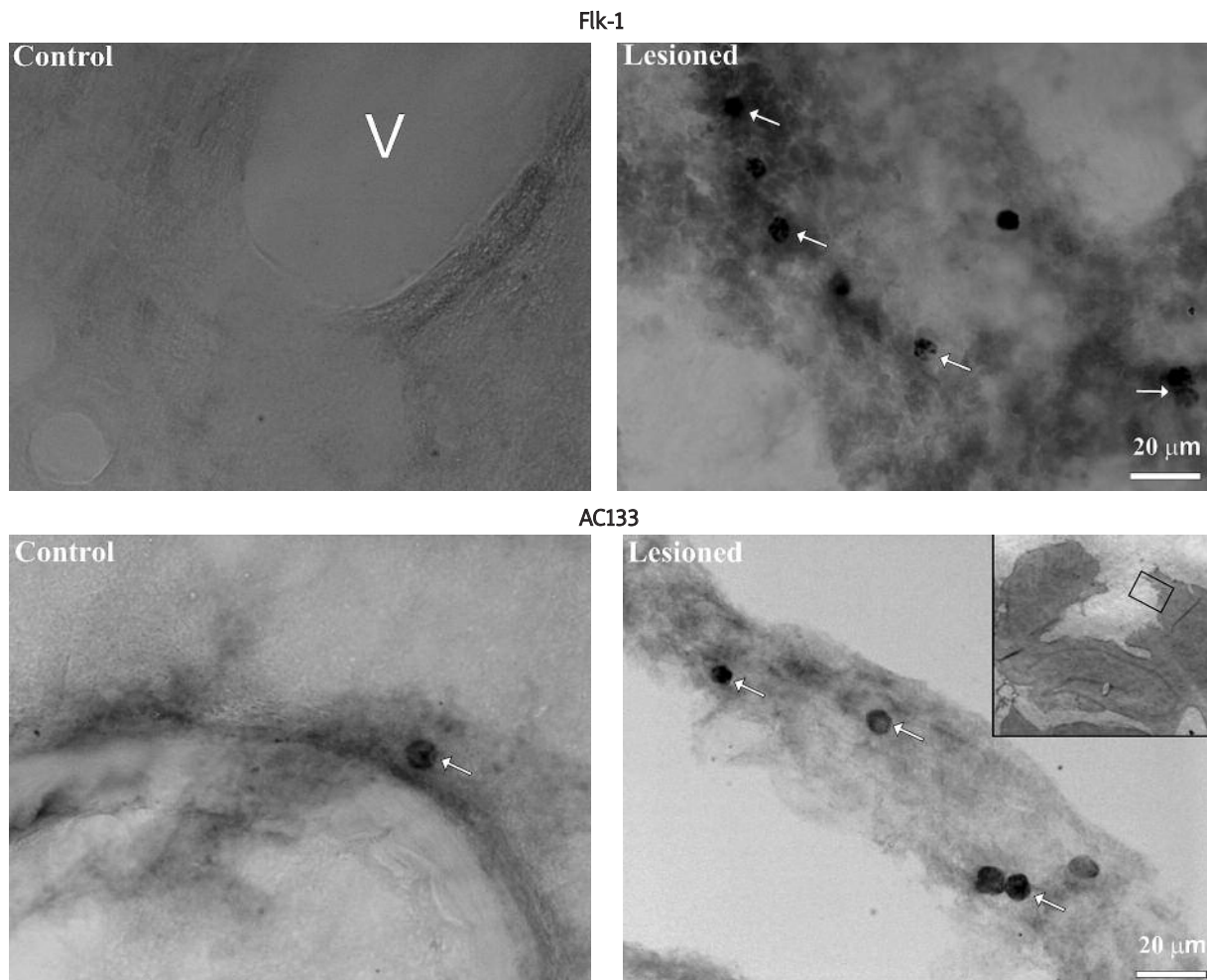


Fig. 9. Flk-1 and AC133 immunoreactivities in cerebral cortex from control (left panels) and lesioned (right panels) rats, 4 days post-SBI. Blood vessel (“V” in the upper right quadrant of panel A denotes the lumen of a blood vessel) and the adjacent parenchyma from control rats show the presence of no or single positively stained cells, whereas the peri-lesion brain parenchyma from lesioned rats shows the expression of both these markers (arrows) in multiple cells. The insert shown in the lower right panel denotes anatomical orientation of the pictured areas.

that the cells flooded in with extravasated blood. From post-SBI day 4 on, cells of the same morphology were found in the lumen and walls of intact (‘old’) blood vessels as well as in the perivascular space, implying their migration from the blood. The phenotype of the presumed EPC has been verified both by Flk-1 (VEGF receptor) and AC133 immunohistochemistry (Fig. 9) and by electron microscopy immunocytochemistry [28,30]. Interestingly, whereas mature endothelial cells are commonly believed to express vimentin, our studies show little or no vimentin immunostaining in mature rat brain vessels (see Fig. 4). Notably, normal rat brain capillaries also show no

presence of Weibel-Palade bodies [64], which are typically found in the endothelium of capillary and non-capillary peripheral blood vessels.

The absence or only marginal expression of cytokeratin in the alleged EPC indicated that they were not, despite a strong expression of vimentin, plain counterparts of embryonic angioblasts. It has also been found by immunohistochemistry that they were highly diversified in terms of AC133 expression, but uniformly showed high expression of Flk-1. Western blotting showed a two-fold increase in Flk-1 immunoreactivity in the peri-SBI region 48 h post-SBI [28,30].

Considerable heterogeneity *in vitro* of human EPC from circulating blood became the basis for the assumption that various subpopulations of these cells play different roles in angiogenesis [40]. However, the results of our studies indicate that traumatic brain injury mobilizes, for the task of forming new vessels, a rather uniform subset of EPC that show phenotypic diversification due to their gradual maturation. Typically, the formation of mature endothelium takes about two weeks [62].

Expression of metalloproteinases

Post-injury reconstruction/remodelling of the affected brain parenchyma is related to the actions of the metalloproteinases (MMPs) that degrade the extracellular matrix and damage the BBB [3,16,84]. Data from both human clinical and experimental animal studies revealed increased expression of these proteases, particularly MMP2 and MMP9, suggesting a role for these enzymes in mobilizing EPC after SBI [82,86] and likely also after brain infarct. Our studies have clearly shown the presence of the two MMPs in the vessels in the peri-SBI region. Both in the vessel walls and in the parenchyma of this zone, there were cells expressing both the markers of immature endothelial cells (Flk-1 and AC133) and MMP2 as well as MMP9; ultrastructurally, these cells also showed the presence of characteristic 5-8 nm diameter fibrils. The presence of AC133/MMP2 and Flk-1/MMP9 double-positive cells that were structurally unrelated to the vessels was also evident in the brain parenchyma in the close vicinity of the SBI zone [29]. These data document the presence of the two MMPs in the EPC-like cells that form new capillaries after surgical injury to the cortex, and suggest that the elevations in MMP2 and MMP9 in these cells in the vicinity of the SBI are directly related to the post-injury remodelling of the cortex, and particularly to the formation of new blood vessels.

Neuronal reaction

Beginning 24 hours after the SBI, the cortex surrounding the injury site showed the presence of necrotic neurons and, particularly at later time points, of neurons with ultrastructural and light microscopy signs of apoptosis [74], which constituted the great majority of this cell type in the affected region (of about 1 mm thickness). As the tissue decayed and phagocytes removed the debris, the zone of genera-

lized neuronal death moved deeper and deeper into the brain parenchyma. It is known that neuronal death caused by mechanical brain injury or ischaemia depends considerably upon excitotoxicity and the associated upsurge in free radical formation [36,39]. Studies on the expression of proteins involved in the initiation, regulation and promotion of apoptosis have demonstrated enhanced expression of the pro-apoptotic Bax protein and the main protease of the apoptotic cascade in mammalian cells, caspase-3, in degenerating neurons. There is also an emphasis on the role of these proteins in SBI-induced death of cortical neurons [13,15,74].

Our studies indicate that some neurons in the immediate vicinity of the dying ones in the peri-SBI region do survive despite being supposedly within the reach of the same injury-related death-promoting factors. A distinguishing mark of the surviving neurons was high Flk-1 immunoreactivity [30], which was in line with the finding of a positive correlation between the presence of Flk-1 and cell survival after various brain insults [66]. The simultaneous expression of Flk-1 in the endothelium of new capillaries and neurons in the peri-SBI region supports the hypothesis that assumes a role for VEGF in synchronizing the neuronal survival-promoting response with the formation of new blood vessels in this zone [66].

Astrocytic reaction

Beginning on post-SBI day 2, the cortex around the SBI showed an enhanced astrocytic reaction (astrogliosis and hypertrophy), but no dying astrocytes were seen this early after the trauma. These observations did not exclude (because of the well-known problems with identifying such cells, see [68,78]) the possibility that the injury induced some immediate astrocytic degeneration and apoptosis. Dying astrocytes (Fig. 10) and astrocytes with ultrastructural abnormalities were apparent in the scar region beginning one month post-SBI, and the range of abnormalities widened with the passage of time (*cf.* Figs. 6 and 10). Six to 12 months after the SBI, intracellular deposits with ultrastructural characteristics of amyloid fibrils were also found at the perimeter of the expanding cavity (see Fig. 11), whereas no such fibrils were seen in age-matched intact controls.

Most recent data from the literature indicate that astrocytes play a pivotal role in the delayed post-traumatic disintegration of brain parenchyma [43,80]. This suggestion is in line with the results of

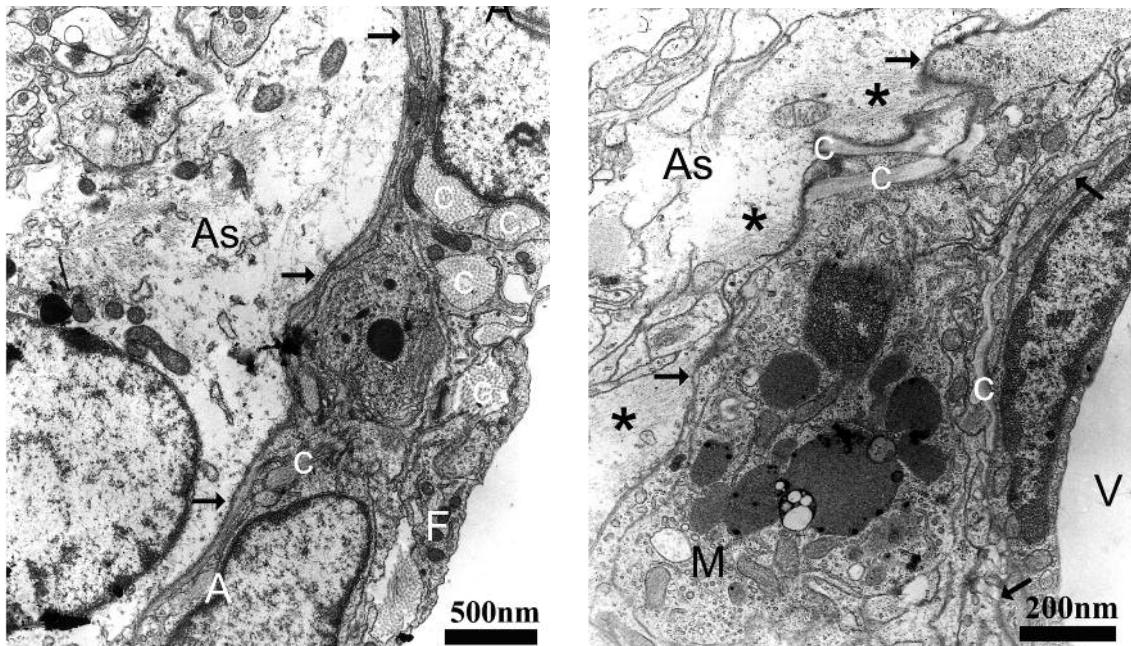


Fig. 10. Left micrograph shows the boundary of the glial scar, 3 months post-SBI. A swollen (necrotic) astrocyte (As) is separated from astrocytes of normal appearance (A) by basement membrane-like material (arrows). The scar abounds in collagen fibres (c) consisting of multiple fibrils. F – a collagen-producing fibroblast-like cell. Right micrograph shows a blood vessel and perivascular space in the glial scar region, 3 months post-SBI, showing oedematous necrotic astrocytes (As) and a macrophage (M) located between basement membrane lamellae (arrows). GFAP fibrils and collagen fibres are marked, respectively, with asterisks and “c”s.

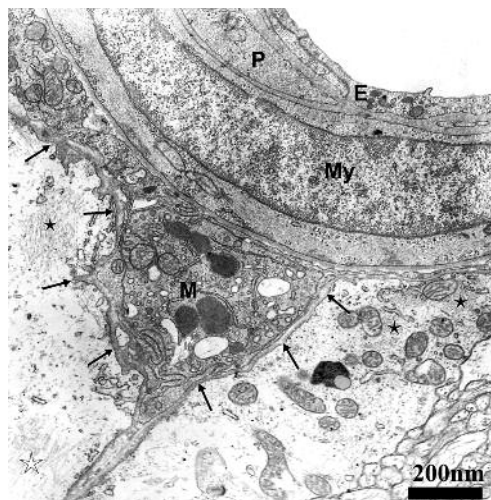


Fig. 11. Lesioned hemisphere cortex precapillary vessel, 12 months post-SBI. The abluminal side of the basement membrane (arrows) encompassing the smooth muscle layer of the vascular wall shows the presence of aggregated fibrils with ultrastructural characteristics of amyloid (black asterisks). M – macrophage, P – pericyte, E – endothelial cell, My – myocyte. Large white asterisk denotes glial fibrillary acidic protein fibrils.

studies from our laboratory [31]. Among other things, it has been postulated that the MMPs secreted by astrocytes are the cause of the depletion of oligodendrocytes and myelin that occurs at later times after cortical injury, and of the consequential white matter damage [80]. It has also been suggested that the MMPs destroy the repair enzymes present in neuronal nuclei [83], a phenomenon which may be one of the causes of apoptosis of the cells. It is also well documented that glial cells are of importance for the well-being and survival of neurons after brain injury [20]. All said, the intensity and duration of astrogliosis are of great importance for the consequences of brain trauma, but we are still far from full elucidation of the underlying mechanisms.

Role of macrophages

The material studied showed substantial infiltration of macrophages into the brain parenchyma from the SBI site. There is considerable support in the literature for an essential role of these cells in the induction of an immune reaction in a variety of

situations that impair CNS functioning, e.g. after brain stroke [17,45,48]. The intensity and duration of the SBI-induced inflammatory reaction are of key importance for the outcome of the attempted repair as well. In the presented model, macrophages were initially present only at the perimeter of the injury. In the wound, they were located in the vicinity of blood vessels, and in the border zone they formed – together with astrocytes – the cell groups penetrating the developing glial scar [31]. Later on, they were also seen between the lamellae of the basement membrane of ‘old’ vessels (see Fig. 10, lower panel).

Relevance to human clinical situations

The studies presented above demonstrated that the formation of the glial scar did not mark an end to the reconstruction/remodelling of the SBI zone and its vicinity. The brain region encompassing the SBI site was always subject to secondary damage resulting in brain parenchyma loss that extended far beyond the primary injury. A similar phenomenon has been reported in a variety of other animal models of focal brain insults, including closed head injury [42,54]. There is no hard proof that exactly the same phenomena also take place in a human clinical setting. Nevertheless, the present results may have some relevance to human clinical settings involving surgical and other types of brain trauma. There are a number of long-term human studies demonstrating progressive atrophy of various brain structures after traumatic brain injury [10,76], stroke [24,35,72], surgical treatment of aneurysmal subarachnoid haemorrhage (e.g. see [8]) and therapeutic lobectomy in epilepsy patients [25,71]. Notably, the post-stroke alterations were reportedly related to the development of microangiopathy in at least some cases [24].

Concluding remarks

Formation of the post-SBI glial scar is associated with widespread angiogenesis that takes place both proximally and at a distance from the injury. The distance has a bearing both on the mechanism of new capillary formation and on the structure of the new vessels. The course of the reconstruction and possibly also the further fate of the SBI zone may depend on the abnormal (?) angiogenesis promoted by the participating EPC. There are grounds for doubt regarding the beneficial role of the alleged EPC in

post-trauma brain healing, despite the fact that this role has been commonly postulated (e.g. see [73]).

Considering the postulated key role of the NVU in brain functioning, one may suspect that destabilization of the glial scar and the continuing loss of brain parenchyma are linked to defects of some new capillaries. The latter include endothelial hyperplasia, faulty basement membrane and the absence of pericytes that are supposedly indispensable for appropriate angiogenesis and capillary function [22]. These abnormalities may result in NVU dysfunction and contribute to the postinjurious progressive brain atrophy [10]. The possible significance of the EPC and lipofibroblast-like cells for these processes needs further studies.

The possibility of the formation of novel NVUs and possible irregularities in their setup and functioning, as well as the formation of pathological protein deposits, also concerns brain trauma due to other factors, e.g. interstitial haemorrhage or excitotoxicity. Especially interesting seems the relationship – for which there is considerable clinical and experimental support – of these phenomena with neurodegenerative disorders [4,88].

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