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ORIGINAL ARTICLE

The changes in the ultrastructure of the cerebrovascular junction after traumatic injury of the cerebral cortex in rats

Michal Walski & Barbara Gajkowska
Laboratory of Cell Ultrastructure, Medical Research Centre, Polish Academy of Sciences

Correspondence to: Michal Walski, Ph.D., D.Sci.
Laboratory of Cell Ultrastructure, Medical Research Centre,
Polish Academy of Sciences, Pawiński ST. 5 02-106 Warsaw, Poland.
E-MAIL: puun@cmdik.pan.pl

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Abstract

OBJECTIVES: The effect of the traumatic injury of the cerebral cortex on the ultrastructure of the cerebrovascular junction was studied in rats. The aim of the present study is to describe the ultrastructural alterations in the cerebrovascular junction in rat cerebral cortex after traumatic injury. We were particularly interested in the alterations in endothelium, pericytes and the differentiated population of cerebral macrophages.

MATERIAL AND METHODS: The observations were conducted four days (group I-five animals) and seven days (group II–five animals) after induction of cortical trauma. Traumatic injury was induced in the fronto-temporal region of cerebral cortex in general anesthesia with 20 mg/kg ketamine hydrochloride.

RESULTS: In the first group we found the features of damage of the blood-brain barrier and migration of the morphological blood components to the perivascular space. The trauma caused necrosis and apoptosis within brain tissue. An important observation was the presence of numerous brain macrophages that participated in phagocytosis of damaged cellular elements. Additionally, we found an increase in the connective tissue ground substance around brain capillaries. In the second experimental group we noted an increased number of pericytes (1–3) near capillary walls. In some instances, the basement membrane surrounding the pericytes was interrupted and these cells were also located beyond the rim of the vessel wall. Some pericytes showed numerous phagolysosomes indicating that these cells belonged to perivascular macrophages. Moreover, we observed a population of phagocytes residing in close contact with neurons. These cells were different from the typical perivascular macrophages.

CONCLUSIONS: These observations indicate that the traumatic injury of the brain results in mobilization of a heterogeneous population of brain macrophages. This study indicates that different subpopulations of macrophages emerge in the region of traumatic brain damage, and that the morphology and dynamics of these phagocytes changes and depends on the time elapsed after the initial traumatic incident.

Introduction

Brain injury caused either by mechanical trauma or focal or global ischemia may result in the impairment in the protective role of brain-blood barrier [1]. Depending on the degree of injury, several protective mechanisms are activated which leads to the partial regeneration of brain parenchyma but may also result in additional injury via necrosis or apoptosis of the neurons [2]. There are numerous studies on the role of glia in the prevention of neuronal death and the maintenance of the homeostasis within the perivascular space in traumatized brain [3]. The reactive hyperplasia and hypertrophy of astrocytes are common phenomena taking place in the central nervous system following traumatic tissue destruction. Moreover, impairment of the blood-brain barrier opens a venue for the influx of blood cells, especially the mononuclear phagocytes [4, 5].

Activated brain macrophages and microglia play an important role in the pathogenesis of acute cerebral events. Research showed that microglia plays an active role in the evolution of brain injury after ischemia and mechanic trauma [6]. Thus, the microglial cells are involved in phagocytosis of damaged neuronal elements, antigen presentation and initiation of immune responses and production of factors causing further damage of the tissues [7, 8]. The question of the origin of differentiated cerebral phagocytes and their relationship to microglia and the undifferentiated perivascular cells is a subject of active research [9, 10]. Phagocytic macrophages appear in large numbers at the sites of brain injury. Although these brain macrophages are supposedly derived from circulating monocytes, the relationship between the macrophages and reactive microglia has not been fully elucidated [11].

The aim of the present study is to describe the ultrastructural alterations in the cerebrovascular junction in rat cerebral cortex after traumatic injury. We were particularly interested in the alterations in endothelium, pericytes and the differentiated population of cerebral macrophages.

Material and Methods

Wistar rats of a mean weight of 200 g were used. Traumatic injury was induced in the fronto-temporal region of cerebral cortex in general anesthesia with 20 mg/kg ketamine hydrochloride. The wound was sutured and dressed under aseptic conditions. Four or seven days after the procedure (experimental groups I and II, respectively) the brains were perfused via the left heart ventricle with 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, at 20°C. Tissue section were fixed in the same solution for 20 h and postfixed in 1% OsO_4 and 0.8% K_4FeCN_6. The material was dehydrated in alcohol gradient, embedded in Spurr resin, cut into ultrathin sections and analyzed in JEM-1200 EX electron microscope.

Results

Material was sampled from the cerebral cortex of rats subjected to mechanical brain trauma in the fronto-temporal region. The animals were divided into two groups representing the changes at the 4th and 7th day after trauma, respectively.

The cerebrovascular junction four days after trauma

In the luminal vascular space red blood cells, leukocytes and thrombocytes were present in the vicinity of the endothelial surface. In some instances the cytoplasmic processes of the endothelia separated blood cells from the main lumen of the vessel (Fig. 1). These "pockets" formed discrete compartments containing also fragments of damaged cells and organelles and the fine fibrillar clot (Fig. 2). The tight junctions between endothelial cells were often morphologically changed and channels were formed between the cytoplasmic processes of endotheliocytes forming shunting pathways for cell passage from the vascular lumen to the perivascular space (Fig. 3).

Erythrocytes were present in the perivascular space facing brain parenchyma and inside the perivascular phagocytes. Moreover, phagocytized cells and differentiated lysosomal vacuoles filled with membrane fragments and lipid globules were present in morphologically differentiated populations of macrophages (Fig. 4). In the perivascular space we observed the multiplication of basal membrane, single collagen fibrils and fine fibrils comprising the proteoglycan aggregates. Importantly, apoptotic cells with characteristically condensed chromatin were present in the perivascular space (Fig. 5). In the nuclei of glial cells the chromatin was condensed into chromatids suggesting vigorous cell proliferation but we not able to correlate this feature with a particular subtype of glial cells (Fig. 6).

The cerebrovascular junction seven days after trauma

Platelets were present inside the vessels but we did not observe any leukocytes. The endothelial cytoplasmic processes were often long and protruded towards vessel lumen. Numerous pericytes were present in the abluminal space facing brain parenchyma (Fig. 7). These cells exhibited well developed and numer-

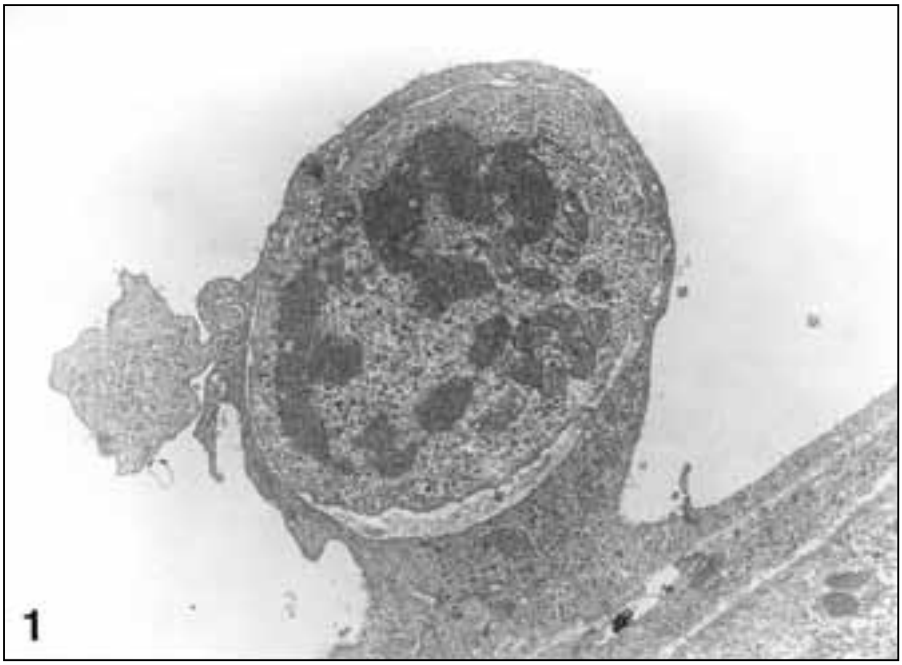

Fig. 1. Experimental group I. Note a leukocyte surrounded by cytoplasmic processes of the endothelial cells in brain capillary. x12000.

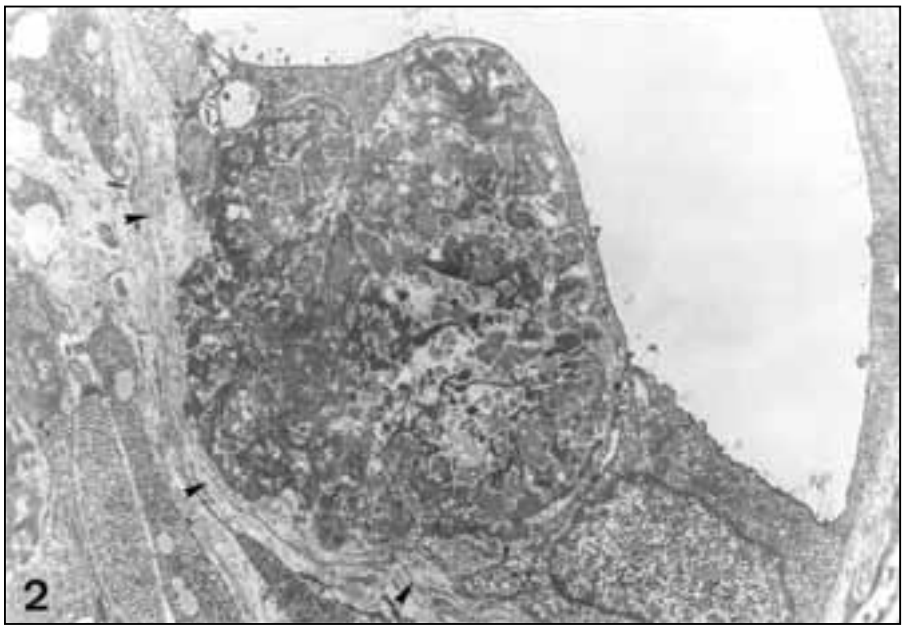

Fig. 2. Experimental group I. Pocket in the endothelium containing fine fibrillar material and cellular debris. Multiplicated basement membrane at the abluminal side of the vessel (arrowheads). x12000.

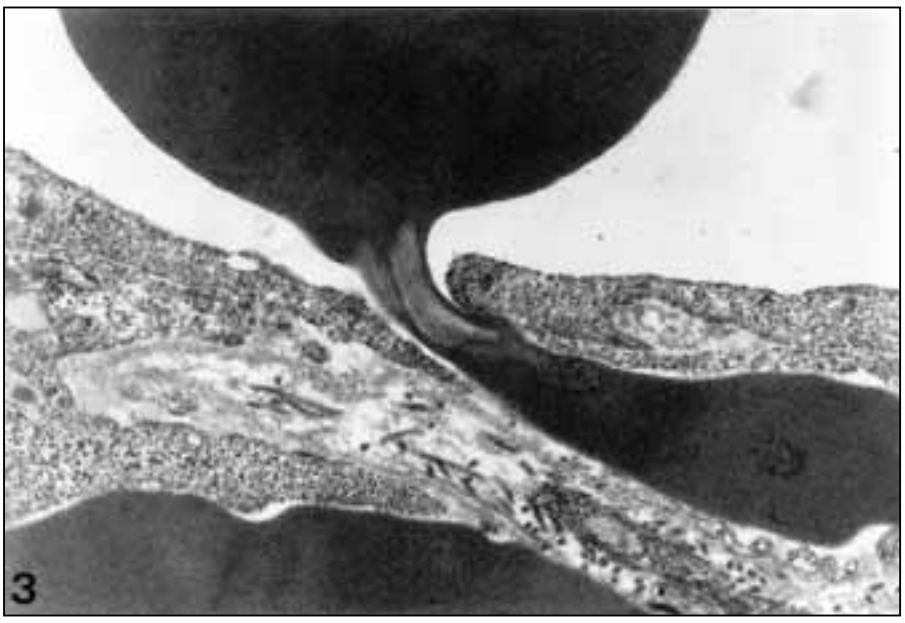

Fig. 3. Experimental group I. Erythrocytes present under the endothelium and in the widened space between endothelial cells. x25000.

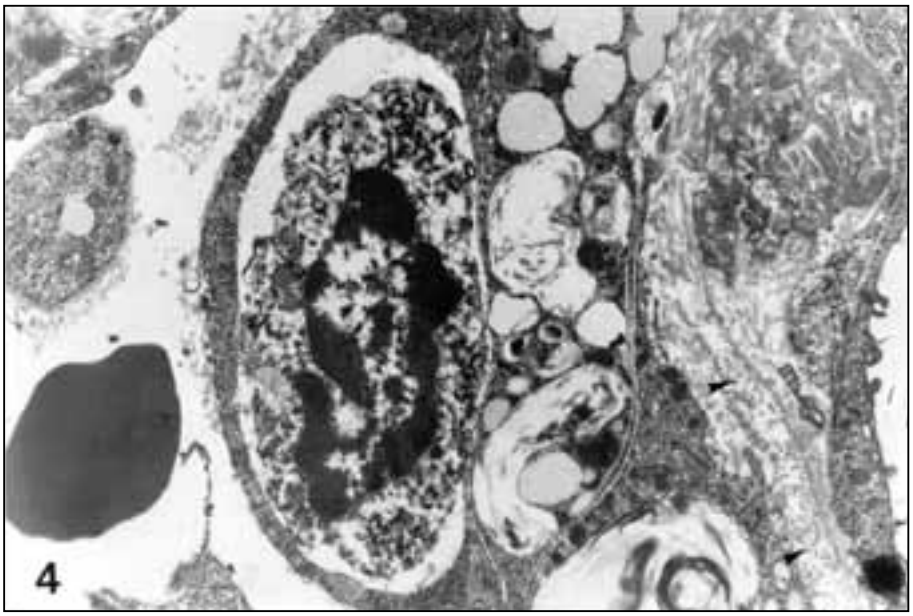

Fig. 4. Experimental group I. Perivascular space with macrophages loaded with phagocytosed material. Note necrotic cell inside one of the macrophages. Arrowheads indicate abundant subendothelial extracellular matrix. x12000.

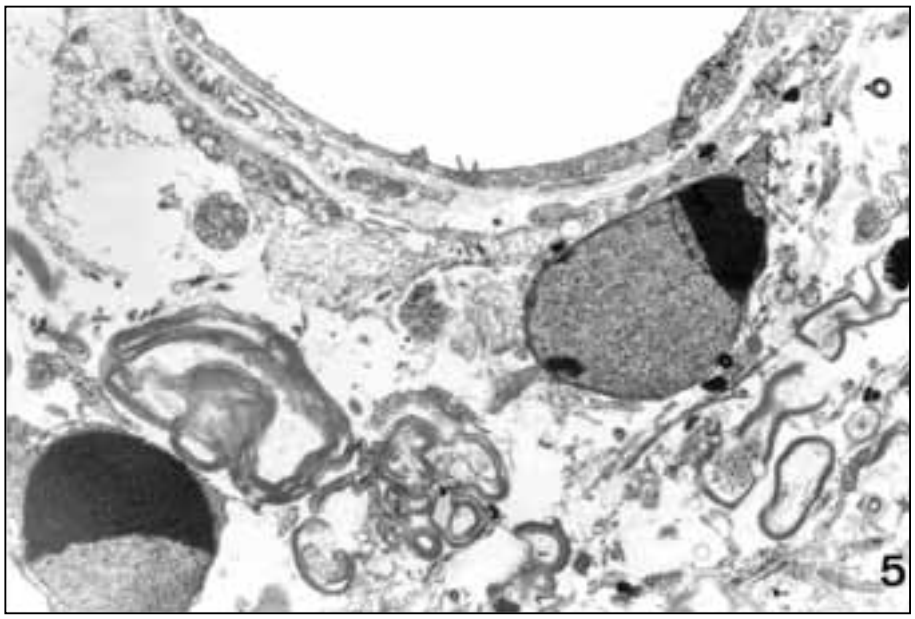

Fig. 5. Experimental group I. Two apoptotic cells with characteristic chromatin aggregation near the capillary vessel. x10000.

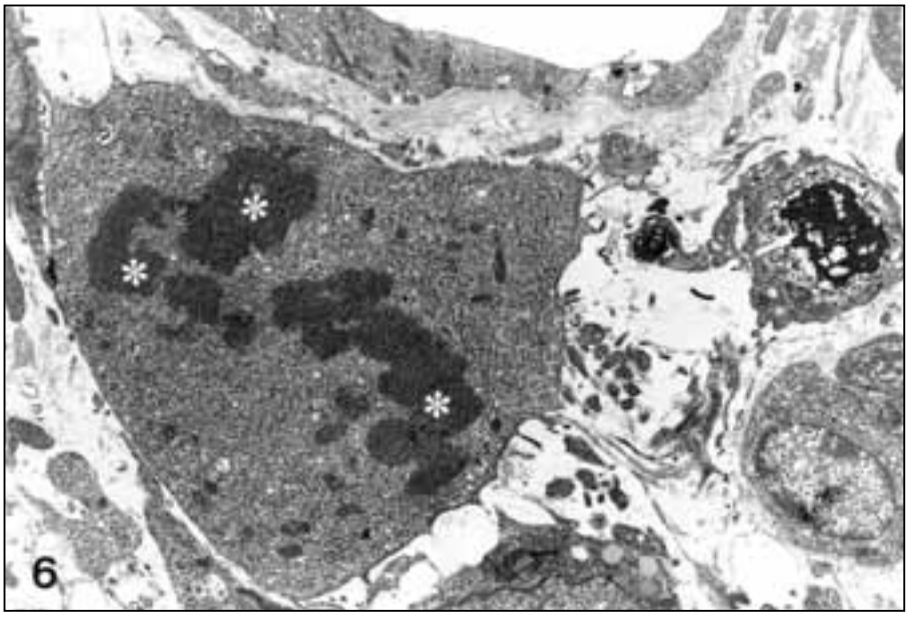

Fig. 6. Experimental group I. Note a cell with mitotic figures (white asterisks) and an apoptotic cell (white arrows) inside a phagocyte. Numerous collagen fibrils in the intercellular space. x6000.

ous organelles, especially the morphologically differentiated lysosomes and dense bodies.

The pericytes and endothelial cells were surrounded by a widened layer of basement membrane. Apart from the homogenous matrix, single collagen fibrils were present between the layers of basement membrane.

Morphologically differentiated phagocytes, resembling the macrophages comprised the most abundant population of cells in the perivascular space. Inside these macrophages we detected numerous dense bodies and phagosomes filled with lipid globules and myelin-like bodies. The phagocytes were located directly under the endothelial layer and were partially or fully surrounded by the basement membrane (Fig. 8). The macrophages localized beyond the basement membrane adhered to neurons with the cytoplasmic processes. In the perinuclear area of these cells we noted differentiated phagosomes and the centrosomes with the tubules of the mitotic spindle (Fig. 9).

Discussion

Traumatic injury of the brain initiates several interrelated processes, involving not only resident parenchymal and vascular cells but also infiltrating inflammatory cells. Angiogenesis, reactive gliosis, migration of inflammatory cells into the central nervous system are triggered by brain trauma [7, 12, 1]. Giordana et al (1994) and Fujita et al (1998) performed monoclonal antibody stainings of brain sections after stereotactic cortical injury [13, 7] and showed the presence of proliferating cells in the brain. These cells were absent six hours after trauma, increased in quantity during three days and subsequently diminished in number during the next two weeks. Our studies demonstrated an aggregation of leukocytes at the endothelia and the passage of inflammatory cells from blood to brain parenchyma four days after cortical injury. Caceres et al (1995), [14] observed leukocyte accumulation at plasma membranes of damaged endothelial cells after ischemia and reperfusion. These authors propose that ischemic injury promotes interactions between endothelia and leukocytes rather that a selective damage of intercellular junctions between endothelial cells. Probably, these increased leukocyte-endothelial cell interactions are caused by increased expression of adhesion molecules and integrins by these cells [12, 5], which leads to the migration of inflammatory cells through vessel wall to perivascular space and further to brain parenchyma. Likewise, we observed passage of erythrocytes to the subendothelial space through widened tight junctions. A similar process has been proposed to exist in bacterial meningitis and in reperfusion injury [15, 16].

The tight junctions formed by the membranes of endothelial cells are highly dynamic structures and can transiently increase the permeability of brain capillaries [4]. The transmembrane protein constituents of tight junctions can be posttranslationally modified in the physiological and pathological states affecting the blood-brain barrier [15, 17]. Our observations indicate that opening of these junctions may constitute the venues for the passage of leukocytes from the blood to the perivascular space where the blood-borne monocytes act as brain macrophages.

Four days after trauma we observed that the different morphological forms of these cells formed cytoplasmic processes surrounding with the fragments of damaged brain tissue. Numerous phagosomes filled with the damaged subcellular fragments were present inside these phagocytes. Fujita et al (1998) [13] showed that between the third and fifth day after cortical injury in the rat, the number of the cells showing surface phenotype of macrophages increase whereas the number of these cells decrease after the seventh day after injury. According to these authors, the observed brain macrophages originate from the circulating monocytes rather than from the resident precursors in brain parenchyma. In this early phase after brain injury it is possible to detect the blood-bone cells and the necrotic and apoptotic glial cells and the neurons.

Brain ischemia or cerebral trauma is considered to cause a secondary brain damage caused by brain edema, impairment of the blood-brain barrier and exposure of the perivascular brain tissue [6]. Long-term ischemia and trauma may cause directly necrosis of cerebral neurons. The cellular debris is subsequently removed by cerebral macrophage [7, 18].

Apoptosis takes also place after brain injury; in this study we observed single apoptotic cells in the perivascular area. Apoptosis is considered to constitute a delayed and selective process leading to cell elimination from the tissues [1, 19]. In contrast to necrosis, apoptosis may affect scattered individual cells rather than clusters of cells, entire tissue or organ compartments [20, 3]. Apoptosis takes also place in cells whose genetic material has been altered or damaged by exogenous factors, such as hypoxia, hyperoxia or other factors inducing neurodegeneration [18, 21]. These factors induce changes in protein expression leading to activation of endonucleases fragmenting DNA and causing chromatin rearrangement. Resulting apoptotic bodies have a very short half-life in the range of hours and are therefore difficult to detect by morphologic methods [1, 22].

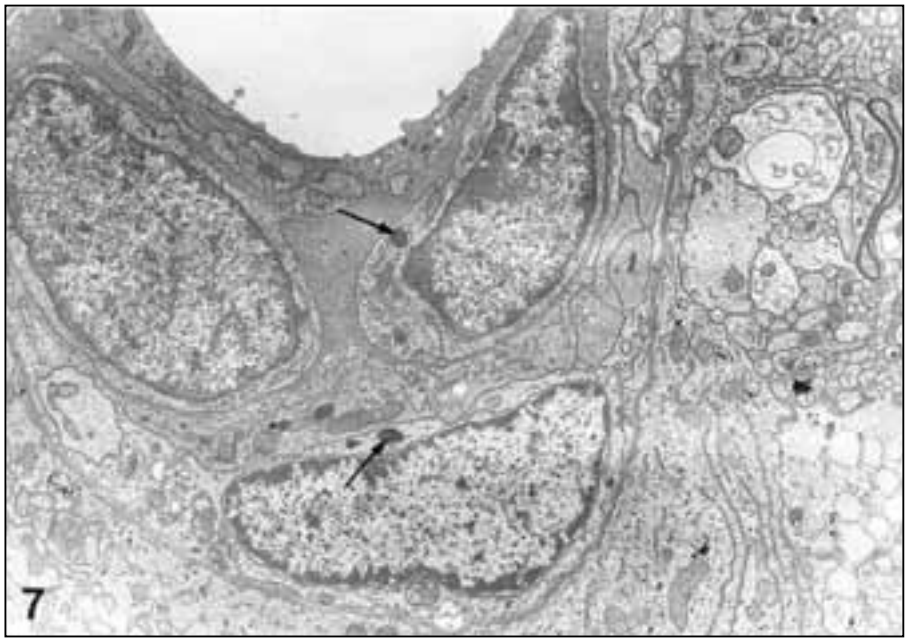

Fig. 7. Experimental group II. Several pericytes surrounded by basement membrane at the rim of the capillary. Arrow – dense bodies. x8000.

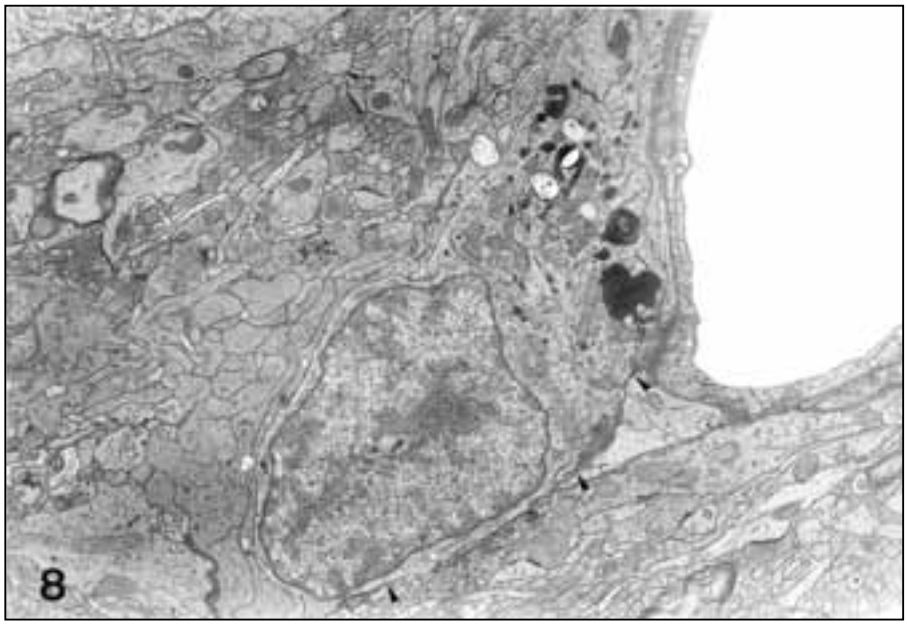

Fig. 8. Experimental group II. Perivascular phagocyte surrounded by basement membrane (arrowheads). Note active Golgi and numerous phagolysosomes in the perikaryon. x8000.

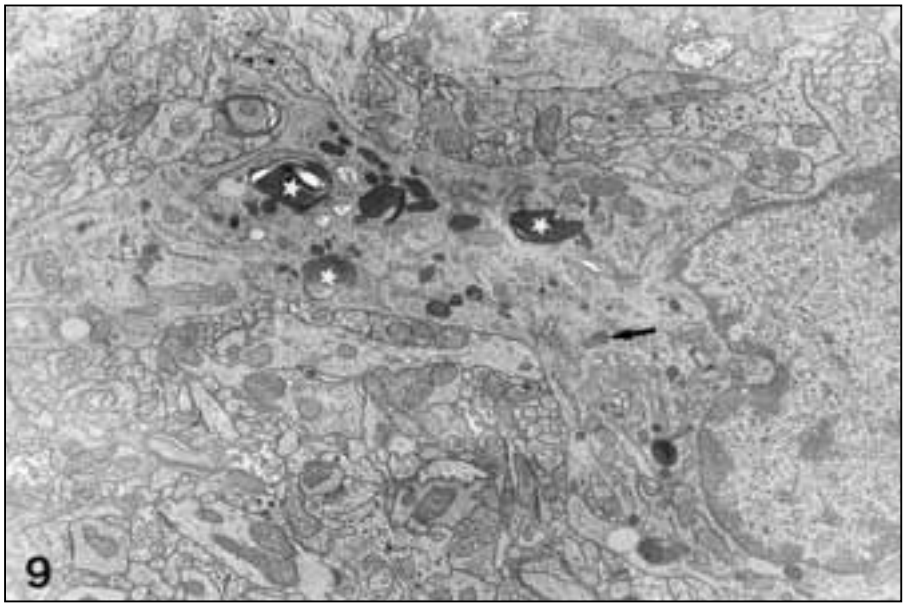

Fig. 9. Experimental group II. Cerebral macrophage containing dense bodies and phagolysosomes, well-developed Golgi and the mitotic spindle (arrow). x10 000.

In summary, we detected several alterations in the perivascular region of the cerebral cortex four days after the traumatic injury of the brain. In particular, we noted proliferation of cerebral macrophages, necrotic and apoptotic cell death and features of regenerative cellular proliferation.

Seven days after cerebral trauma we were unable to detect any features of blood cell migration to the perivascular space or any alterations in the tight interendothelial junctions. The pericytes in the perivascular space were increased in number and there was an increase in the quantity of connective tissue, namely the proteoglycans and the collagen fibrils.

The pericytes are generally accepted as being of mesodermal origin [23] and these cells are supposed to contribute to the synthesis of the protein component of the extracellular matrix of blood vessels [23, 24]. These cells may synthesize collagen fibrils in the perivascular space [25]. Our earlier studies showed the process of collagen formation in cerebral perivascular space in rat brain after transient global ischemia or during aging [26, 27]. Numerous studies suggested brain pericytes as a source of macrophage activity since they are capable of phagocytosis and antigen presentation [10]. These perivascular macrophages may leave the perivascular space, cross the basement membrane and migrate into brain parenchyma [28]. The source of these cerebral phagocytes, blood monocytes or ameboid microglia, is still a matter of debate [7, 29].

In the material sampled seven days after trauma the macrophages were loaded with phagosomes and were often in close contacts with neurons. Fujita et al. 1998 showed an increase in microglial cells between the seventh and fourteenth day after brain injury [13]. Based on these findings, these authors consider two morphologically different subpopulations of cerebral mononuclear phagocytes.

Both macrophages and microglial cells can be versatile effector cells when activated and are likely to mediate brain damage through several mechanisms [3, 9, 29]. Oehmichen et al (1999) and Ziaja, Janeczko (1999) suggested that the microglial reaction is a general phenomenon associated with traumatic brain injury [30, 31]. Our present studies demonstrated the impairment in the brain-blood barrier in the early posttraumatic period, which was associated with the migration of cells from the blood to the perivascular space. The consequences of this pathological process comprised necrosis and apoptosis in the perivascular space. This study indicates that different subpopulations of macrophages emerge in the region of traumatic brain damage, and that the morphology and dynamics of these phagocytes changes and depends on the time elapsed after the initial traumatic incident.

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