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Study of restorative processes in brain laceration in the first seven days after traumatic brain injury

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Abstract

Traumatic brain injuries represent the main cause of death and invalidity all over the world. Persons surviving a severe traumatic brain injury often present long-term disabilities, sensitive and motor deficits, cognitive, vegetative or mental disorders. Brain injuries are directly caused by the traumatic agent, and indirectly caused by the action of cells involved in the restorative process. The main cells involved in the restorative process are microglias and astrocytes. By using an experimental model, we investigated the reaction of these cells in the first week after a severe brain injury, followed by brain laceration. Of the two cell types, the most rapid and intense reaction was held by the macroglias, also known as resident macrophages of the central nervous system. Alongside the activation of local microglias, in the restorative process there were also involved blood monocytes that turned into macrophages. 24 hours after the injury, the number of macrophage cells/mm^2 at brain wound level increased 2–4 times, after three days – 10–12 times, and after seven days – over 20 times. The astrocyte reaction was slower, their activation being signaled no sooner than three days from injury, when their number in the perilesional brain parenchyma increased approximately two times, while after seven days – approximately 4–5 times. Both astrocytes and macrophages (microglias), besides their beneficial effects in restoring traumatic brain injuries, may have unfavorable effects upon the nervous cells in the immediate proximity of the injury. Destruction of vascular network by the traumatic agent, and the extremely slow restore of vascularization, partially explain brain neurons death on extend areas.

Keywords: traumatic brain injury, cerebral laceration, gliosis, microglia.

Introduction

Traumatic brain injuries (TBI) represent the main cause of morbidity, death and invalidity at worldwide level [1–3]. Every year, all over the world, there are recorded a few million brain injuries, of which 1.5 million patients die [4]. Only in the United States of America, in the adult population, there are recorded about 155 000 cases of traumatic brain injuries and approximately 12 000 deaths having the same cause every year [5]. In children and young population less than 19-year-old, brain injuries are even more frequent. According to some authors [6], in the emergency rooms all over the USA there present about 630 000 young patients every year, of whom cca. 60 000 require hospitalization and about 6000 brain-injured patients subsequently die.

In Europe, the rate of annual incidence regarding the patients admitted to hospital with TBI is approximately 235 in 100 000 individuals, and mortality has an average rate of approximately 15 in 100 000 individuals [7]. In Great Britain and France, brain injuries represent 15–20% of deaths in individuals aged between 5 and 35-year-old [8]. According to some studies, until 2020 TBI will become one of the main causes of morbidity and death [9]. TBI are largely caused by car accidents, work accidents, wars, violence or sports [10].

Of all traumatic brain injuries, the most severe form is cerebral laceration, characterized by the nervous system damage, on more or less extended areas, caused by the penetration of a foreign object into the brain parenchyma (projectile, white weapon, bone fragments), or by a violent impact between the skull and a contusive object. Laceration is more severe than brain contusion, due to the fact that, besides the brain parenchyma damage, there also appear leptomeninges ruptures (pia mater and arachnoid) [11].

Healing of post-traumatic brain injuries is quite difficult and, most of the times, it aggravates neuron death and exacerbates neurological deficits, by developing an excessive local inflammatory reaction. Brain inflammation caused by TBI may have both negative and positive effects [12, 13]. That is why it represents a therapeutic target to minimize the negative and neurotoxic effects, and to promote the beneficial and neutrophic effects, at the same time.

In our study, we proposed to investigate, in the experiment animal, the involvement of microglias and astrocytes in the healing process of brain parenchyma caused by severe traumatic injuries associated with brain tissue laceration, as these cells are directly involved in the post-traumatic healing of brain injuries.

Materials and Methods

In order to perform the study, we selected a number of 15 adult common Wistar rats, with a weight between 270 and 310 g, from the Animal Facility of the University of Medicine and Pharmacy of Craiova, Romania. Both before and during the experiment, the animals were held in standard conditions of light, temperature and humidity, and had unlimited access to food and water (*ad libitum*). Artificial light provided a cycle period of 12 hours light/ 12 hours darkness. The special environment conditions (temperature, humidity, air exchanges) were provided by complex air conditioning equipment and central heating of the animal facility.

For performing the experiment, we obtained the approval of the Ethics Board of the University of Medicine and Pharmacy of Craiova, according to the European Council Directive 11.24.1986 (86/609/CEE), the European Convention regarding vertebrate animal protection (2005) and Govern Ordinance No. 37/02.02.2002.

Animal preparation

In order to perform the traumatic brain injury, we performed the anesthesia of laboratory animals, through the intraperitoneal administering of a Ketamine hydrochloride mixture (Ketalar®, Parke-Davis) 100 mg/kg and Xylazine hydrochloride (Rompun®, Bayer) 20 mg/kg. Before starting any invasive surgical procedure, the anesthesia depth was monitored after 10 minutes since the anesthetic peritoneal injection, through the defense reflex produced by the hallux pinching.

The anesthetized animals were placed on an electrical pillow for maintaining a constant body temperature during the entire surgical intervention.

Surgical intervention

The skin covering the skull of the animal was first disinfected, by using an alcoholic iodine solution, followed by a longitudinal incision until a good surgical area was achieved, where both parietal bones, sagital, coronary and lambdoid skull sutures could be visualized. The entire subcutaneous tissue covering the skull was removed with a scalpel. After highlighting the skull bones, by using a dental drill there was performed a craniotomy window, 2 mm long and 1 mm wide, in the right parietal bone, 1 mm aside and parallel to the sagital suture, and 1 mm rostral from the lambdoid suture. During craniotomy, there were taken breaks at every 15–20 seconds, in order to prevent bone heating and subsequent generation of caloric injuries upon the brain parenchyma. For a better and faster cooling, the drill was introduced in a 0.9% sodium chloride solution, for several times. When the window depth reached the internal lamina of the right parietal bone, this remaining intact, there was produced a penetrating traumatic brain injury, 5 mm deep, thus ensuring the dura mater rupture and brain tissue laceration at parietal lobe level in the right hemisphere, using a sterile contusive instrument, made of stainless steel, 2 mm long and 0.5 mm in diameter. After performing the brain injury, the skin was sutured, and the incision margins were sterilized with a iodine alcoholic solution. The animals did not undergo any other surgical procedures, and they were monitored every day.

After performing the brain injury, the animals were divided into five groups of five animals each that were sacrificed after one, three and seven days since injury. Then, for encephalon harvesting, there was again performed the anesthesia of animals from every group, with 10% Ketamine (60 mg/kg) and 2% Xylazine (10 mg/kg), followed by a paraformaldehyde injection into the aorta, for the primary encephalon fixation. Taking into consideration the reduced consistency of the encephalon, there was performed the brain box dissection, followed by the encephalon harvesting with maximum care.

The harvested biological material was then fixed in 10% neutral formalin for 72 hours at room temperature, and included in paraffin, using the classical histological technique.

The sectioning of the biological material was performed in the rotary microtome Microm HM350, equipped with a water bath section transfer (STS, Microm). For the histological study, there were performed 4 μm thick sections, stained with Hematoxylin–Eosin (HE). For the immunohistochemical study, the histological sections were collected on poly-L-Lysine covered blades, and dried in a thermostat at 37^0C for 24 hours. Then, the sections followed the classical protocol: deparaffinization and hydration. For antigen demasking, the blades were boiled in a sodium citrate solution, pH 6, for 21 minutes (seven cycles of 3 minutes each) in a microwave oven. After blade boiling and cooling, they were washed in tap water and distilled water for 15 minutes. The endogen peroxidase blocking was performed by blade incubation in 3% oxygenated water for 30 minutes, at room temperature, followed by distilled water washing for 10 minutes, and a 1% phosphate-buffered saline (PBS) solution washing for 5 minutes. After that, there followed the non-specific sites blocking, using 2% skim milk for 30 minutes. The sections were then incubated with primary antibodies, for 18 hours (over night), in a refrigerator at 4^0C. The next day, there was applied the biotinylated secondary antibody for 30 minutes, at room temperature, followed by 1% phosphate-buffered solution washing (three baths of 5 minutes each), followed by Streptavidin-HRP for 30 minutes at room temperature and blade washing in 1% PBS, 3×5 minutes. The signal was detected by using the 3.3'-Diaminobenzidine (DAB) (Dako) and the reaction was stopped in 1% PBS. There followed the Mayer's Hematoxylin contrasting, alcohol dehydration, xylene clarification and blade fitting by using a DPX environment (Fluka).

In our study, we used the following markers (Table 1):

Table 1 – ***Antibodies used for the immunohistochemical study***

Antibody	Code	Clone	Antigen retrieval	Specificity	Dilution	Source
Anti-Iba1	ab5076	Polyclonal	Sodium citrate buffer, pH 6	Microglia/macrophages	1:1000	Abcam

Antibody	Code	Clone	Antigen retrieval	Specificity	Dilution	Source
Anti-GFAP	M0761	6F2	Sodium citrate buffer, pH 6	Astrocytes	1:2000	Dako
Anti-CD34	ab81289	EP373Y	Sodium citrate buffer, pH 6	Vascular endothelium	1:100	Abcam
Anti-Neu N	ab128886	Polyclonal	Sodium citrate buffer, pH 6	Neuronal	1:1000	Abcam

Results

The traumatic brain injury performed by us caused the destruction of the leptomeninges, neurins and neuronal extensions, glial cells and blood vessels, through shearing, rupture or extension mechanisms of the brain tissue components. The injury damaged an area of the parietal gray matter, cortical white matter and hippocampus, thus generating a longitudinal cavity with irregular walls, due to laceration.

After 24 hours since injury, there could be observed the accumulation of a high blood quantity in the remaining cavity, especially in the deep part of the wound, because of the brain vascular network damaging (Figure 1). Hemorrhagic foci were also observed at perilesion level, sometimes quite remote from the damaged area, up to 1–2 mm (Figure 2). These hemorrhagic foci were indirectly caused, because of the pressure produced by the mechanic factor upon the entire brain hemisphere, thus causing vascular wall rupture, because that brain vessels have a very thin wall, hardly resistant to mechanic traumatic injuries, with little collagen fibers in their structure.

The neuronal distress appeared clear after 24 hours since injury. Around the wound, the neurons presented a multitude of morphological changes, from cellular necrosis processes, with "neuronal ghosts", nucleus condensation, cytoplasmic vacuolization, up to neuronal ischemia phenomena (red neurons). As foreseen, the most serious morphological changes of the neurons appeared around the wound. The cellular lesions diminished in intensity as the distance from the injured area became more remote.

Neuronal distress, namely brain cell degeneration induced by the traumatic factor, was much better expressed by the use of the anti-NeuN antibody (neuronal nuclear antigen). After 24 hours, there could be observed an important diminishing of the immunohistochemical reaction in the neurons situated at perilesional level, in comparison to the contralateral hemisphere (Figures 3 and 4). Neuron death in the immediate proximity of the lesion was produced because of the anoxia onset, through suppression of the brain blood flow.

Astrocytes, specifically highlighted through the use of anti-GFAP antibody (glial fibrillary acidic protein), were almost entirely damaged in the lesional area, and at perilesional level they were partially altered by the accumulation of extravasated blood from blood vessels or by perilesional edema occurrence. Due to this reason, at perilesional level, astrocytes appeared altered, fragmented, with a heterogeneous distribution, in comparison to their aspect in the left hemisphere (Figures 5 and 6).

Macrophage system cells were the first ones reacting to the traumatic aggression, after 24 since injury. Resident macrophage microglias of the brain are normally found in a small number, both in the gray matter and in the white matter. They are difficult to notice due to the small cellular body and quite delicate extensions (Figure 7). In our experiment, in the contralateral hemisphere of the injury, the number of microglia was approximately 60–75 cells/mm^2 of gray matter. Instead, at perilesional level, there appeared numerous round, large sized, intensely anti-Iba1 reactive cells (Figure 8). Their number varied between 154–320 cells/mm^2 of gray matter, representing a 2–4 times growth compared to the ordinary microglia number. A careful examination of the injured area allowed us to observe that the macrophage system cells present at post-traumatic brain injury come both from the microglia activation in the brain tissue, and from the monocytes in the blood. Some monocytes present in the blood vessels around the wound appeared intensely Iba1-reactive, which indicates that, because of the traumatic injury, there appeared local factors entering blood vessels and activating monocytes in order to enhance the cellular phagocytosis mechanisms (Figures 9 and 10).

The microvascularization was severely altered in the immediate proximity of the brain injury. The number of capillaries was much smaller than the one in the normal brain hemisphere, the anti-CD34 antibody being strongly diminished. Moreover, some blood capillaries appeared colabated, with perivascular edema, while others were fragmented, with a discontinuous wall, leading to blood extravasation (Figures 11 and 12). If in the left hemisphere, in the gray matter, the number of capillaries varied between 280–330 vessels/mm^2, in the right hemisphere, in the immediate proximity of the injury, the number of identified capillaries varied between 160–190 vessels/mm^2.

After three days since injury, the neuronal necrosis area was much more extended, with a missing anti-NeuN reaction on extended areas at perilesional level (Figure 13). This microscopic aspect shows the extension of the perilesional ischemia. In the wound wall, there were identified numerous mononuclear cells with a round nucleus. The immunohistochemical reaction to GFAP and Iba1 showed the presence of numerous intensely reactive astrocytes (Figure 14) and of macrophage cells. The number of perilesionally activated astrocytes increased up to 380–440 cells/mm^2, while in the contralateral hemisphere, their number was only 160–210 cells/mm^2, and the macrophage number increased up to 780–860 cells/mm^2 of cortical surface.

In the perilesional area, by using the anti-CD34 antibody, there were identified numerous angiogenesis capillaries, and isolated CD34-positive cells, as well (Figure 15). Also, there were identified remaining, budding blood vessels, where the endothelial cells presented a large ovalary nucleus, similar to some angioblasts (Figure 16). These microscopic aspects lead us to the consideration that the angiogenesis processes have their origin in the endothelial cells of the remaining vessels, but they can also emerge from young CD34-positive cells that may subsequently become angioblasts.

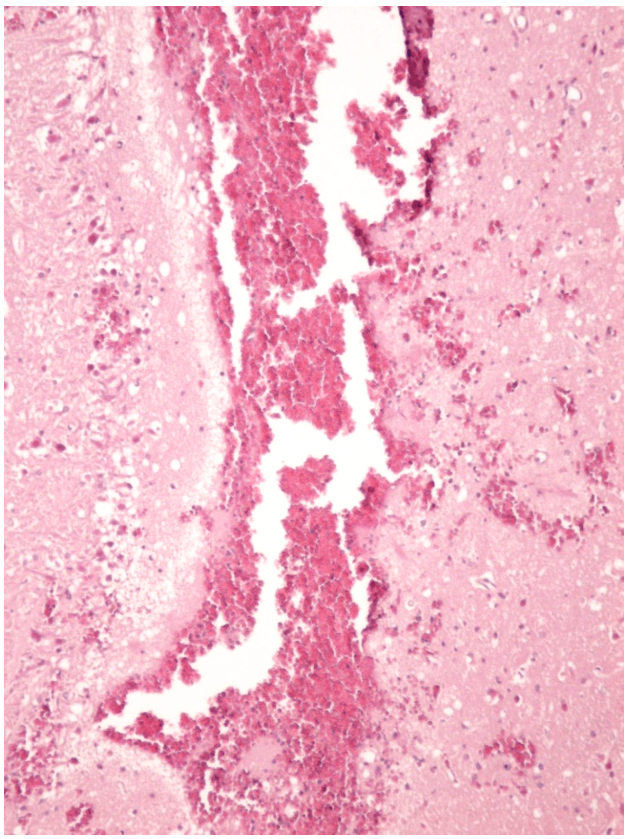

Figure 1 – ***Microscopic image of the brain laceration area after 24 hours since traumatic injury. There may be seen the formation of a cavity with irregular walls, full of blood, because of blood vessels damage in the traumatized area. HE staining, ×100.***

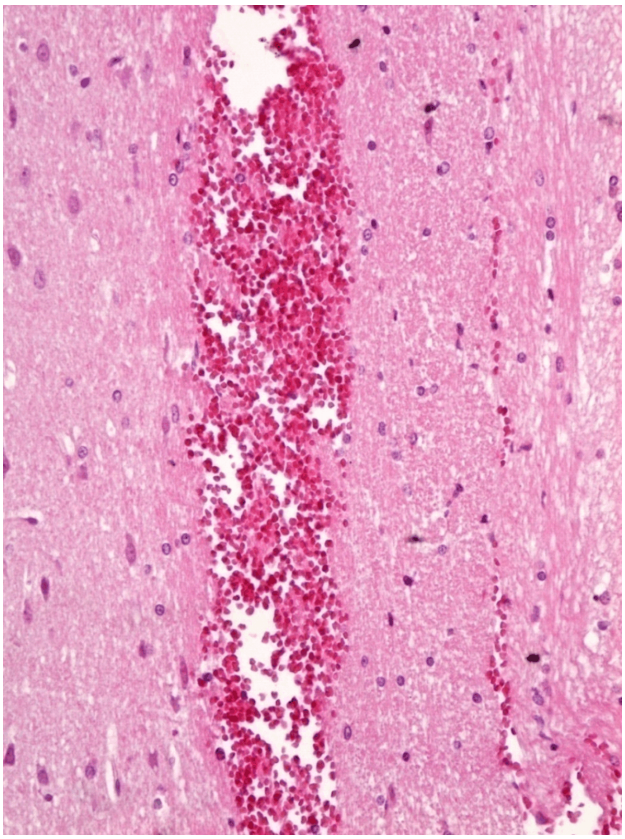

Figure 2 – ***Hemorrhagic focus identified quite remote from the mechanic aggression, after 24 hours since injury. HE staining, ×200.***

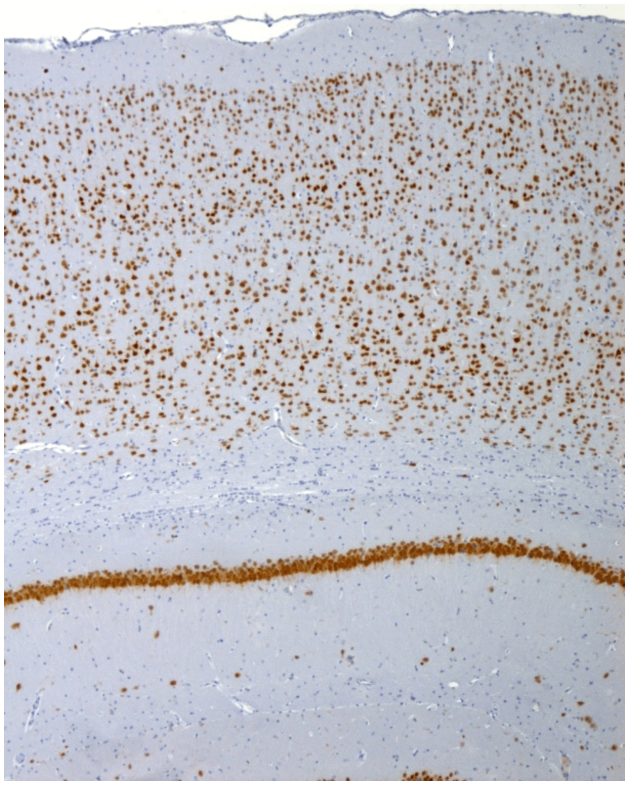

Figure 3 – ***Microscopic image of left brain hemisphere (normal), where we may observe the uniform anti-NeuN reaction of neurons in the brain cortex and hippocampus. Anti-NeuN antibody immunomarking, ×100.***

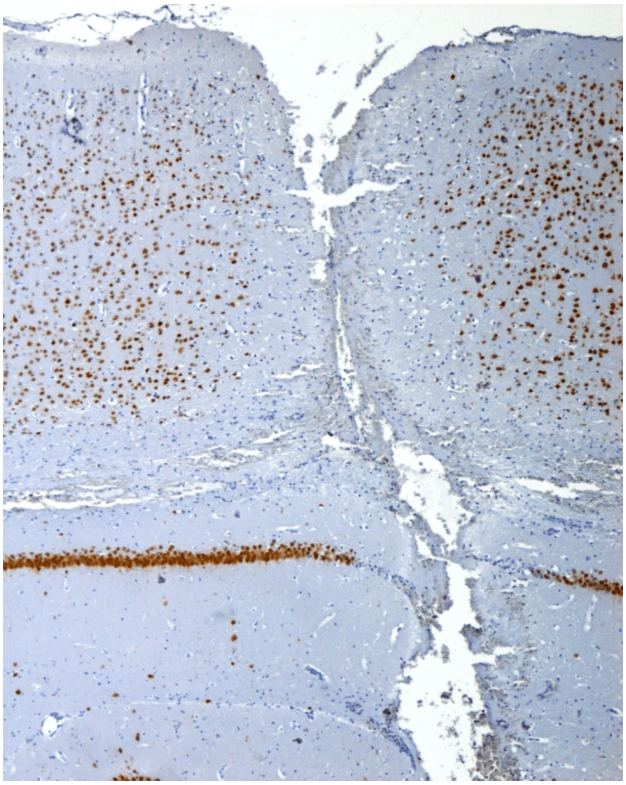

Figure 4 – ***Microscopic image of the right brain hemisphere in the laceration area, where we may observe the absence of perilesional NeuN reaction. Anti-NeuN antibody immunomarking, ×100.***

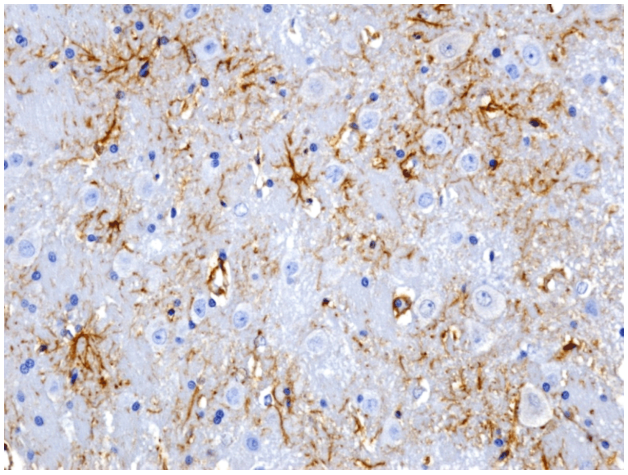

Figure 5 – ***Normal distribution of astrocytes in the brain cortex contralateral to the lesion. Anti-GFAP antibody immunomarking, ×200.***

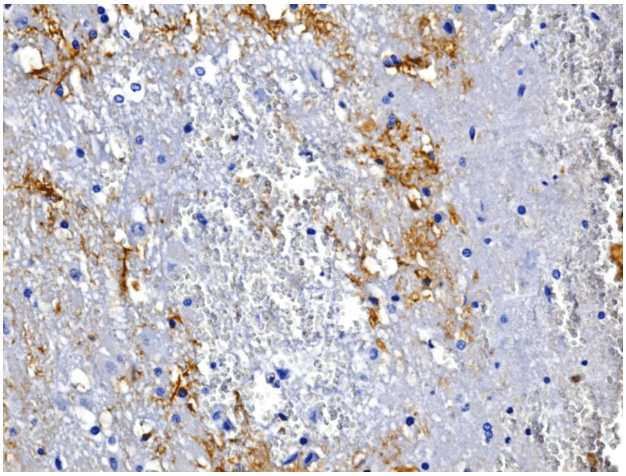

Figure 6 – ***Microscopic image of astrocytes in the immediate proximity of traumatic focus, after 24 hours since injury, where we may observe the astrocyte alteration and a totally heterogeneous distribution. Anti-GFAP antibody immunomarking, ×200.***

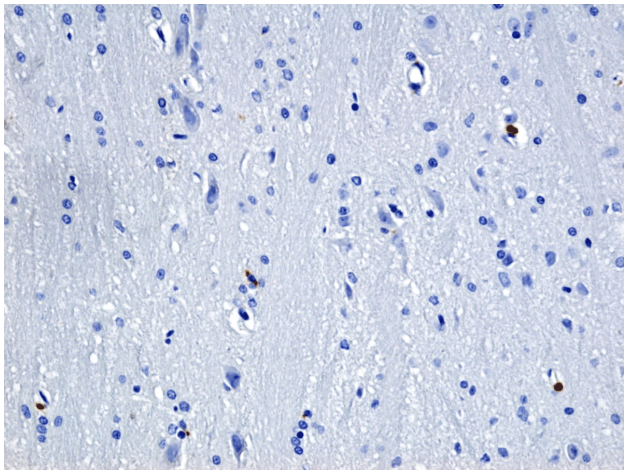

Figure 7 – ***Microscopic image in the brain hemisphere unaffected by the injury, where we may see the presence of some microglias. Anti-Iba1 antibody immunomarking, ×200.***

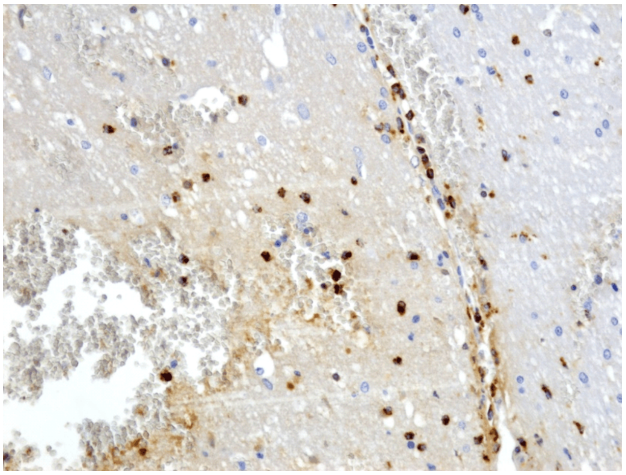

Figure 8 – ***Perilesional area with a large number of macrophage cells in the gray matter. Anti-Iba1 antibody immunomarking, ×200.***

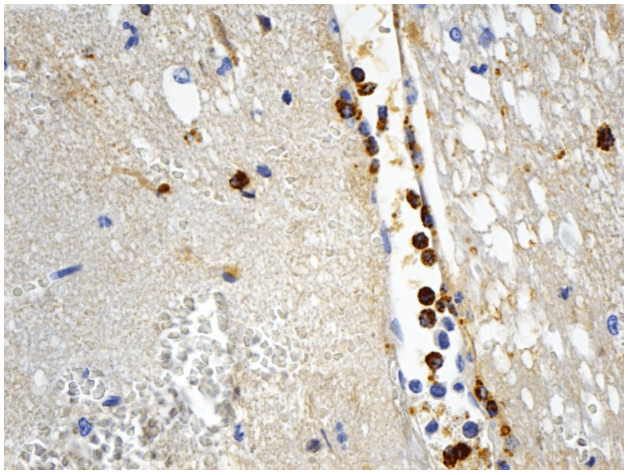

Figure 9 – ***Macrophage cells present both in the gray matter and intravascularly. Anti-Iba1 antibody immunomarking, ×400.***

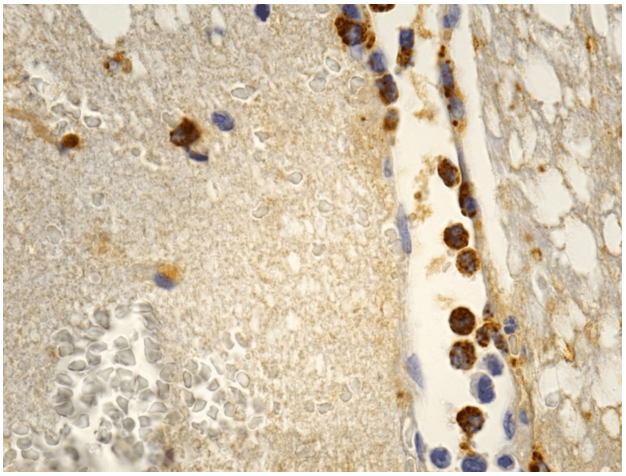

Figure 10 – ***Intravascularly activated monocytes in the immediate proximity of the wound (detail from previous image). Anti-Iba1 antibody immunomarking, ×600.***

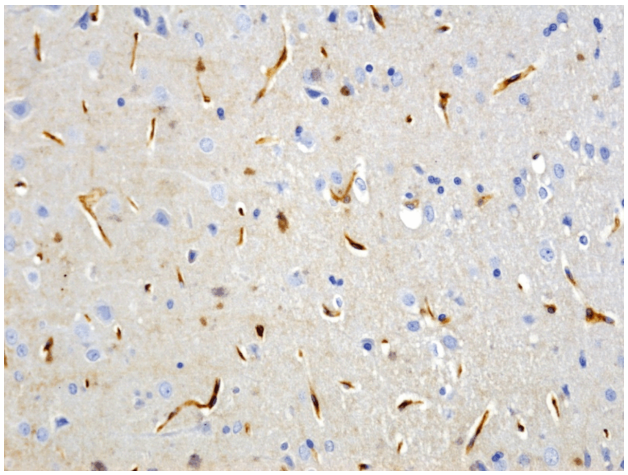

Figure 11 – ***Normal vascular network in the gray matter of the left hemisphere. Anti-CD34 antibody immunomarking, ×200.***

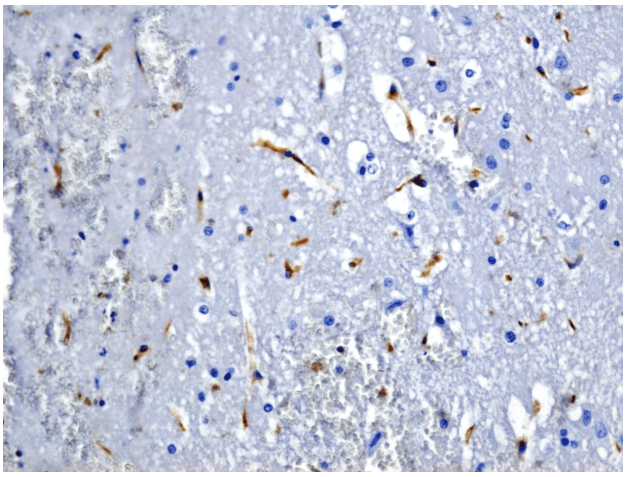

Figure 12 – ***Image of damaged vascular network in the immediate proximity of the traumatic injury. There may be observed the reduction of blood vessels, the colabation of some, with perivascular edema or wall rupture, with blood extravasations in the brain parenchyma. Anti-CD34 antibody immunomarking, ×200.***

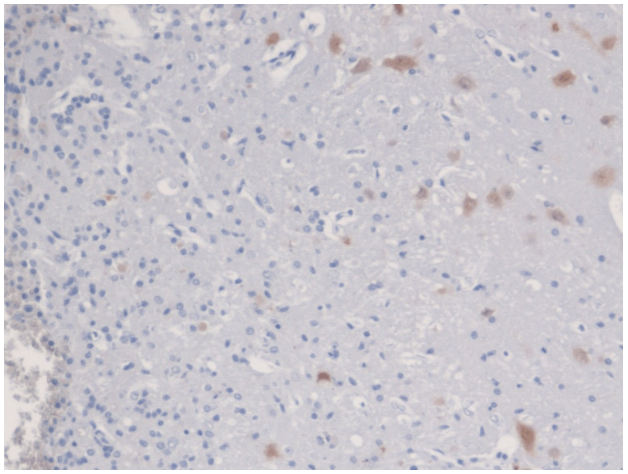

Figure 13 – ***Image of the wound after three days since injury. There may be observed the reduction of anti-NeuN reaction on extended areas, because of neuronal necrosis. Anti-NeuN antibody immunomarking, ×200.***

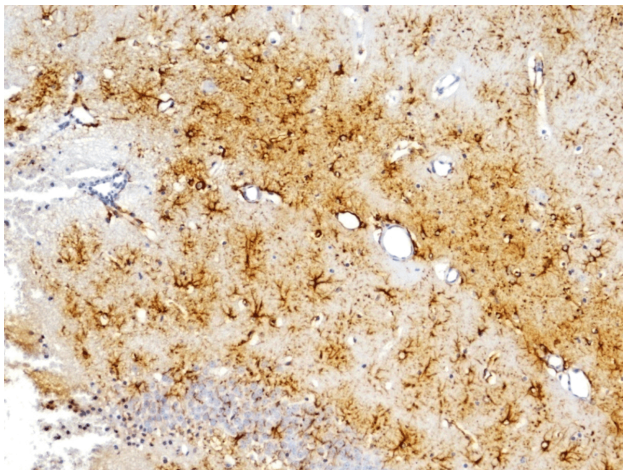

Figure 14 – ***Intense gliosis around the wound after three days since traumatic brain injury. Anti-GFAP antibody immunomarking, ×100.***

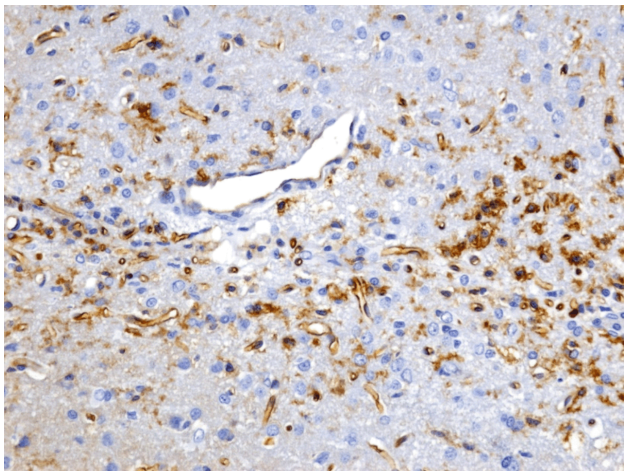

Figure 15 – ***Image from the perilesional area where there are highlighted numerous angiogenesis capillaries with the wall formed of CD34-positive cells. Also, there are numerous heterogeneously disseminated CD34-positive cells in the brain parenchyma. Anti-CD34 antibody immunomarking, ×200.***

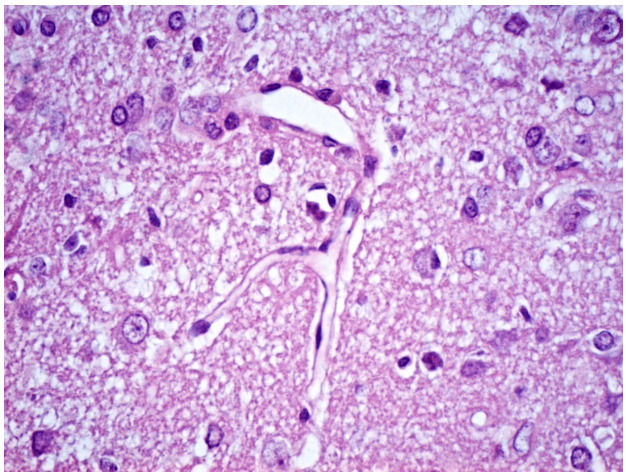

Figure 16 – ***Process of "vascular budding" observed in a capillary adjacent to the traumatic wound. HE staining, ×400.***

After seven days since brain injury, the microscopic study of the histological samples obtained showed that the wound sizes reduced because of numerous mononuclear cells proliferation in the perilesional area. There were also identified areas of small remaining hemorrhagic suffusions and hemosiderinic pigment cells in the cytoplasm (Figure 17). The evaluation of neuron viability by using the anti-NeuN antibody showed us that they presented extended area damage (Figure 18). In the brain parenchyma, there was identified a dense cellular population, with a heterogeneous, scattered arrangement, that overwhelmed the ordinary cellular architecture of the brain cortex. The use of Iba1 and GFAP antibodies showed that, both at wound level, and at perilesional level, there appeared an extremely large number of reactive macrophages and astrocytes (Figures 19 and 20). The density of perilesional reactive astrocytes varied between 480 and 630 cells/mm^2, while, at lesion level, the macrophage cell density was of 1260–1410 cells/mm^2. A small number of reactive astrocytes was identified right in the center of the lesion, which shows the ability of these cells to multiply and mobilize. There could also be observed that, alongside the numeric growth of macrophage cells, there was a change in their morphology, thorough the increase of cytoplasm quantity, nucleus with color diminishing, a fact denoting a looser arrangement of the chromatin, characteristic to young, functionally activated cells.

After seven days since brain injury, the study of angiogenesis processes showed that, both at wound level and at perilesional level, among the astrocytes and macrophages, there developed a well-organized vascular device, with vessels ranging from a few μm up to 45–50 μm in size. Also, there were identified numerous diffusely disseminated CD34 positive cells in the brain parenchyma, at perilesional level that may generate new blood vessels (Figures 21 and 22).

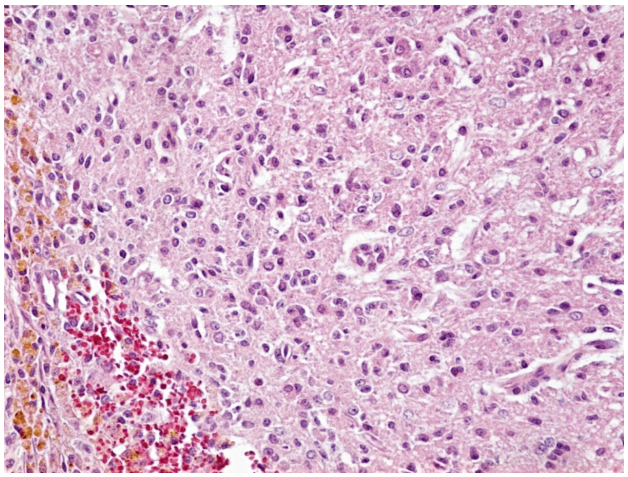

Figure 17 – ***Area of brain cortex after seven days since injury. There may be observed the persistence of some micro hemorrhagic foci and some intracytoplasmatic hemosiderinic pigment cells, with a deep rearrangement of the brain cortex by the proliferation of a mononuclear cellular population. HE staining, ×200.***

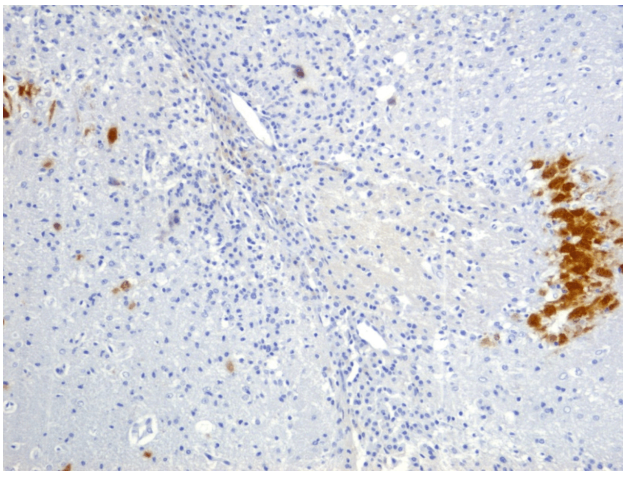

Figure 18 – ***Microscopic image of brain laceration after seven days since injury. There may be seen the absence of neurons on extended areas, both at lesion level and at perilesional level. Anti-NeuN antibody immunomarking, ×200.***

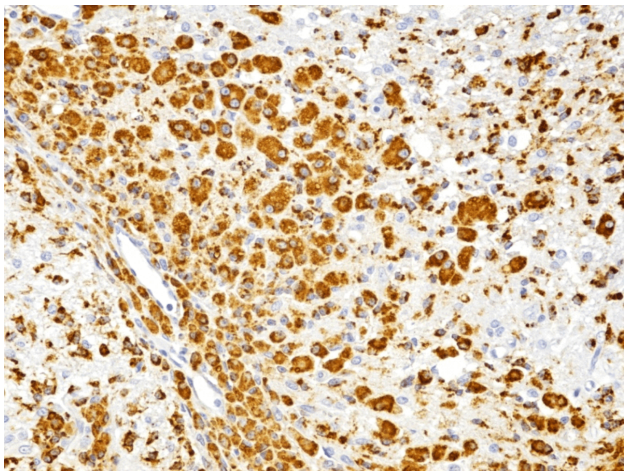

Figure 19 – ***Numerous macrophages accumulated at lesion level, and in the perilesional brain parenchyma, as well, after seven days since injury. Anti-Iba1 antibody immunomarking, ×200.***

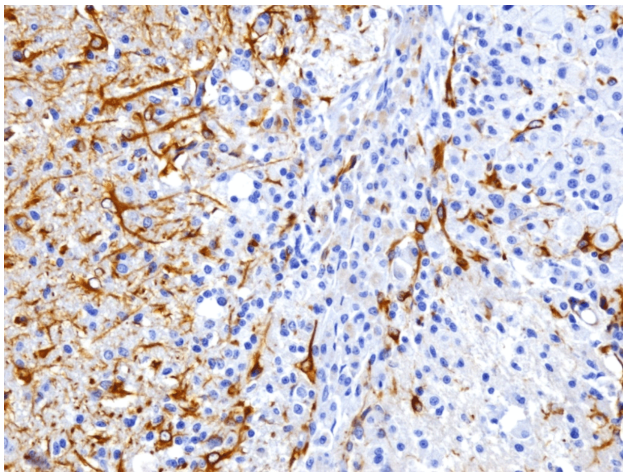

Figure 20 – ***Reactive astrocytes mainly located at perilesional level, after seven days since injury. Anti-GFAP antibody immunomarking, ×200.***

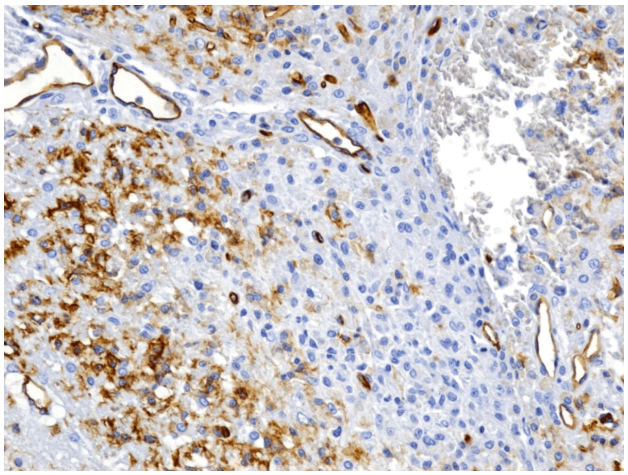

Figure 21 – ***Microscopic image of the lesion after seven days, where we may observe numerous blood vessels in the immediate proximity of the lesion. Also, there are present numerous perilesional diffusely disseminated CD34+ cells, which may generate new blood vessels. Anti-CD34 antibody immunomarking, ×200.***

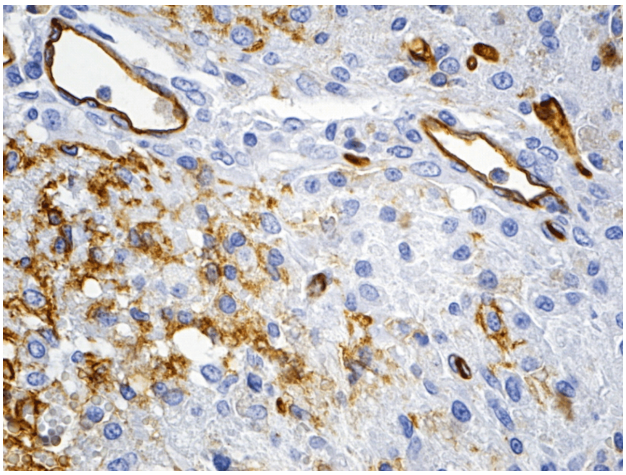

Figure 22 – ***Blood vessels with a well-organized wall, ranging up to 50 µm, in the immediate proximity of the brain injury. Detail from previous image. Anti-CD34 antibody immunomarking, ×400.***

Discussion

Severe traumatic brain injuries immediately cause primary, irreversible lesions, due to the mechanical impact of the traumatic agent upon the brain tissue. These primary lesions start a series of secondary phenomena at cellular, subcellular and molecular level, which may amplify the injury impact upon the neurons and glial cells, resulting in the onset of some very severe clinical signs [14, 15].

The physiopathology of secondary brain lesions is difficult to examine. Most data were given by the experimental models on the experiment animal. Even so, none of the used experimental models did not manage to completely explain the secondary lesions and especially the correlations with neurological and psychiatric pathology present in people with TBI [16].

In our study, we wanted to examine the reaction of microglias, astrocytes and endothelial cells, during the first stages of meningocerebral restorative processes after severe brain injuries, as the intensity of the restorative reactions during the first stages may be correlated with secondary lesions and late brain deficiencies. In our experiment, the traumatic impact caused a brain laceration area, with a diameter of approx. 0.5 mm and a depth of 2 mm. Besides the brain tissue destruction, the injury caused brain vessel destruction on a lot more extended area than the traumatic marking, which led to the emergence of some hemorrhagic foci at quite remote distances from the traumatic site. We consider that these hemorrhagic foci were indirectly caused because of the pressure produced by the mechanic factor upon the entire brain hemisphere. It is well known the fact that vascular lesions, irrespective of their type, cause brain hypoxia, which induces neuron death. Moreover, the accumulated blood as hematomas in the brain parenchyma acts as an exceeding substance in an unexpandable cavity, represented by the brain box, and it determines a "mass effect", thus aggravating the hypoxia caused by the direct vascular network destruction by the traumatic agent.

The local homeostasis disturbance determined a rapid response of the macrophage system cells, characterized through the emergence of Iba1 positive cells, both in the perilesional brain parenchyma and in the brain vessels lumen. We showed that in severe brain injuries there are activated both the microglias, known as resident macrophages of the brain tissue, and the blood monocytes present in the vessels around the lesion. According to some authors [17–19], in a physiological state, microglias, through their extensions, scan the brain parenchyma in order to detect some antigens. After stimulation, microglias change both their morphological aspect and their functional state [20]. They retract their extensions and move towards the damaged area [21], where they release various proinflammatory factors, such as the following cytokines: IL-1beta, TNF-alpha, IL6, prostaglandins, growth factors, nitric oxide or reactive oxygen species [19]. Microglia activation may be performed by the reduction of brain blood flow, accompanied by the onset of brain hypoxia [22, 23]. Apart from microglias, we observed that, in the lesional focus, there migrated numerous blood monocytes that turned into macrophages, without being able to specify which of the two types were predominant. The macrophage number increased over 20 times at brain wound level in the first week after the injury, which denotes the major involvement of these cells in the meningocerebral restorative processes. Numerous studies showed that both microglias and blood macrophages are involved in the phagocytosis processes, thus favoring the tissue restoring and remodeling [24, 25], participate in the fine regulation of the inflammatory reaction [18, 19, 26, 27] and stimulate angiogenesis. We consider that the accumulation of a very high number of macrophages in the brain laceration area, as well as perilesionally, is due to the accumulation of cellular detrituses, because of the traumatic factor acting upon the brain tissue, and also of the presence of numerous microhemorrhagic foci caused by the brain vascular network rupture. Brain hemorrhagic foci cause an additional oxidative stress, through erythrocyte lysis, and hemoglobin and iron release in the brain parenchyma [28–30]. Hemoglobin degradation products constitute a factor that stimulates the phagocytosis activity of the

macrophages. In our study, at brain injury level, we identified numerous macrophages containing a hemosiderinic pigment in the cytoplasm, because of the extravasated red cell phagocytation in the brain parenchyma.

The astrocyte reaction was much slower and less intense, in comparison to the reaction of macrophage system cells. After 24 hours since injury, the astrocyte reaction was absent. Perilesionally, similarly to neurons, astrocytes suffered phenomena of necrosis or autolysis in the first days after the injury. In our study, we observed the presence of a moderate astrocyte reaction, known as reactive glyosis, after three days since injury. It was characterized by the increase of glial cell number on surface unit, increase of cellular volume and glial extensions, increase of the nucleus and cytoplasm quantity and intensification of the anti-GFAP reaction. After seven days since brain laceration, together with the maximum development of the macrophage cells reaction, the astrocyte cells reaction was tremendous.

According to some studies, the astrocyte proliferation is stimulated by the microglia activation, through various cytokines, but especially by the tumor necrosis factor alpha (TNF-alpha) [31–33]. Similar astrocyte reactions were also described in other experimental models [28, 34]. The microscopic aspects observed by us at post-traumatic lesion level lead us to the conclusion that, between the astrocytes and the mcrophage system cells, there are very strong relations in the tissue restoring process, each playing a specific part within this process. Reactive astrocytes have a beneficial effect upon brain lesion restoring, through collecting and eliminating some excessive quantities of neuromediators, especially of glutamate, by restoring the brain blood barrier, as well as by supporting the axonal regeneration [35–37].

Besides the beneficial effects, both astrocytes and microglias may cause pathological effects when cellular proliferation becomes chronic or it is not an excessive one. Chronic microglia activation leads to neuron death and generally induces the neuron degeneration processes [38, 39]. Astrocytes may also aggravate the neuronal lesions, by forming an excessive scar tissue, by secreting inhibitory molecules of the axon regeneration, or by producing proinflammatory cytokines [40, 41]. We consider that, at present, the mechanisms through which these types of glial cells participate in the post-traumatic restorative brain processes are not completely known. Therapeutic modeling of these cells reactivity might have a beneficial effect in the meningocerebral restorative processes.

Conclusions

Severe brain traumatic injuries, which involve brain tissue laceration processes, determine the restorative complex processes where microglias and astrocytes play essential parts. Of the two types of glial cells, the fastest and most intense reaction was held by microglias. In the restorative brain, restorative reaction there also participated blood monocytes that turned into macrophages, their part being of removing cellular debris and blood extravasates resulted from the brain vascular network damage. There cannot be specified the ratio between blood microglias and monocytes in the restorative process of brain injuries. The reaction of macrophage system cells was quite intense, thus after 24 hours since injury, the number of macrophage cells/mm^2 at brain lesion level increased 2–4 times, after three days – 10–12 times, and after seven days – over 20 times. The astrocyte reaction was slower, being signaled only after three days after injury, when their number in the perilesional parenchyma increased approximately two times, and after seven days it increased approx. 4–5 times since traumatic injury. These cells modulation might be a therapeutic target in brain traumatic injuries.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] Bruns J Jr, Hauser WA. The epidemiology of traumatic brain injury: a review. Epilepsia, 2003, 44(Suppl 10):2–10.

[2] Menon DK, Schwab K, Wright DW, Maas AI. Demographics and Clinical Assessment Working Group of the International Interagency Initiative toward Common Data Elements for Research on Traumatic Brain Injury Psychological Health. Position statement: definition of traumatic brain injury. Arch Phys Med Rehabil, 2010, 91(11):1637–1640.

[3] Scholten AC, Haagsma JA, Panneman MJ, van Beeck EF, Polinder S. Traumatic brain injury in the Netherlands: incidence, costs and disability-adjusted life years. PLoS One, 2014, 9(10):e110905.

[4] Opara JA, Małecka E, Szczygiel J. Clinimetric measurement in traumatic brain injuries. J Med Life, 2014, 7(2):124–127.

[5] Richmond R, Aldaghlas TA, Burke C, Rizzo AG, Griffen M, Pullarkat R. Age: is it all in the head? Factors influencing mortality in elderly patients with head injuries. J Trauma, 2011, 71(1):E8–E11.

[6] Vavilala MS, Kernic MA, Wang J, Kannan N, Mink RB, Wainwright MS, Groner JI, Bell MJ, Giza CC, Zatzick DF, Ellenbogen RG, Boyle LN, Mitchell PH, Rivara FP; Pediatric Guideline Adherence and Outcomes Study. Acute care clinical indicators associated with discharge outcomes in children with severe traumatic brain injury. Crit Care Med, 2014, 42(10):2258–2266.

[7] Tagliaferri F, Compagnone C, Korsic M, Servadei F, Kraus J. A systematic review of brain injury epidemiology in Europe. Acta Neurochir (Wien), 2006, 148(3):255–268; discussion 268.

[8] Yang B, Tse KM, Chen N, Tan LB, Zheng QQ, Yang HM, Hu M, Pan G, Lee HP. Development of a finite element head model for the study of impact head injury. BioMed Res Int, 2014, 2014:408278.

[9] Hyder AA, Wunderlich CA, Puvanachandra P, Gururaj G, Kobusingye OC. The impact of traumatic brain injuries: a global perspective. NeuroRehabililitation, 2007, 22(5):341–353.

[10] Sun D. The potential of endogenous neurogenesis for brain repair and regeneration following traumatic brain injury. Neural Regen Res, 2014, 9(7):688–692.

[11] Hardman JM, Manoukian A. Pathology of head trauma. Neuroimaging Clin N Am, 2002, 12(2):175–187, *vii*.

[12] Ramlackhansingh AF, Brooks DJ, Greenwood RJ, Bose SK, Turkheimer FE, Kinnunen KM, Gentleman S, Heckemann RA, Gunanayagam K, Gelosa G, Sharp DJ. Inflammation after trauma: microglial activation and traumatic brain injury. Ann Neurol, 2011, 70(3):374–383.

[13] Kumar A, Loane DJ. Neuroinflammation after traumatic brain injury: opportunities for therapeutic intervention. Brain Behav Immun, 2012, 26(8):1191–1201.

[14] Greve MW, Zink BJ. Pathophysiology of traumatic brain injury. Mt Sinai J Med, 2009, 76(2):97–104.

[15] Schwarzmaier SM, Plesnila N. Contributions of the immune system to the pathophysiology of traumatic brain injury – evidence by intravital microscopy. Front Cell Neurosci, 2014, 8:358.

[16] Morganti-Kossmann MC, Yan E, Bye N. Animal models of traumatic brain injury: is there an optimal model to reproduce

human brain injury in the laboratory? Injury, 2010, 41(Suppl 1): S10–S13.
[17] Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. Science, 2005, 308(5726):1314–1318.
[18] Ransohoff RM, Cardona AE. The myeloid cells of the central nervous system parenchyma. Nature, 2010, 468(7321):253–262.
[19] Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. Physiol Rev, 2011, 91(2):461–553.
[20] Atallah N, Vasiu R, Boşca AB, Creţu DI, Georgiu C, Constantin AM, Sovrea AS. Microglia – performers of the 21st century. Rom J Morphol Embryol, 2014, 55(3):745–765.
[21] Sieger D, Moritz C, Ziegenhals T, Prykhozhij S, Peri F. Long-range Ca^{2+} waves transmit brain-damage signals to microglia. Dev Cell, 2012, 22(6):1138–1148.
[22] Masuda T, Croom D, Hida H, Kirov SA. Capillary blood flow around microglial somata determines dynamics of microglial processes in ischemic conditions. Glia, 2011, 59(11):1744–1753.
[23] Loane DJ, Byrnes KR. Role of microglia in neurotrauma. Neurotherapeutics, 2010, 7(4):366–377.
[24] Colton CA. Heterogeneity of microglial activation in the innate immune response in the brain. J Neuroimmune Pharmacol, 2009, 4(4):399–418.
[25] Cătălin B, Cupido A, Iancău M, Albu CV, Kirchhoff F. Microglia: first responders in the central nervous system. Rom J Morphol Embryol, 2013, 54(3):467–472.
[26] Lambertsen KL, Clausen BH, Babcock AA, Gregersen R, Fenger C, Nielsen HH, Haugaard LS, Wirenfeldt M, Nielsen M, Dagnaes-Hansen F, Bluethmann H, Faergeman NJ, Meldgaard M, Deierborg T, Finsen B. Microglia protect neurons against ischemia by synthesis of tumor necrosis factor. J Neurosci, 2009, 29(5):1319–1330.
[27] David S, Kroner A. Repertoire of microglial and macrophage responses after spinal cord injury. Nat Rev Neurosci, 2011, 12(7):388–399.
[28] Wu J, Hua Y, Keep RF, Schallert T, Hoff JT, Xi G. Oxidative brain injury from extravasated erythrocytes after intracerebral hemorrhage. Brain Res, 2002, 953(1–2):45–52.
[29] Xi G, Keep RF, Hoff JT. Mechanisms of brain injury after intracerebral haemorrhage. Lancet Neurol, 2006, 5(1):53–63.
[30] Cernak I, Wing ID, Davidsson J, Plantman S. A novel mouse model of penetrating brain injury. Front Neurol, 2014, 5:209.
[31] Frei K, Nohava K, Malipiero UV, Schwerdel C, Fontana A. Production of macrophage colony-stimulating factor by astrocytes and brain macrophages. J Neuroimmunol, 1992, 40(2–3): 189–195.
[32] Lee SC, Liu W, Roth P, Dickson DW, Berman JW, Brosnan CF. Macrophage colony-stimulating factor in human fetal astrocytes and microglia. Differential regulation by cytokines and lipopolysaccharide, and modulation of class II MHC on microglia. J Immunol, 1993, 150(2):594–604.
[33] Aloisi F, Borsellino G, Caré A, Testa U, Gallo P, Russo G, Peschle C, Levi G. Cytokine regulation of astrocyte function: *in-vitro* studies using cells from the human brain. Int J Dev Neurosci, 1995, 13(3–4):265–274.
[34] Plantman S, Ng KC, Lu J, Davidsson J, Risling M. Characterization of a novel rat model of penetrating traumatic brain injury. J Neurotrauma, 2012, 29(6):1219–1232.
[35] Frisén J, Haegerstrand A, Risling M, Fried K, Johansson CB, Hammarberg H, Elde R, Hökfelt T, Cullheim S. Spinal axons in central nervous system scar tissue are closely related to laminin-immunoreactive astrocytes. Neuroscience, 1995, 65(1):293–304.
[36] Toz VJ, Doller CM, Malouf AT, Silver J. Astrocyte-associated fibronectin is critical for axonal regeneration in adult white matter. J Neurosci, 2004, 24(42):9282–9290.
[37] Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. Acta Neuropathol, 2010, 119(1):7–35.
[38] Gao HM, Hong JS. Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. Trends Immunol, 2008, 29(8):357–365.
[39] Tansey MG, Goldberg MS. Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. Neurobiol Dis, 2010, 37(3):510–518.
[40] Eddleston M, Mucke L. Molecular profile of reactive astrocytes – implications for their role in neurologic disease. Neuroscience, 1993, 54(1):15–36.
[41] Asher RA, Morgenstern DA, Fidler PS, Adcock KH, Oohira A, Braistead JE, Levine JM, Margolis RU, Rogers JH, Fawcett JW. Neurocan is upregulated in injured brain and in cytokine-treated astrocytes. J Neurosci, 2000, 20(7):2427–2438.

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