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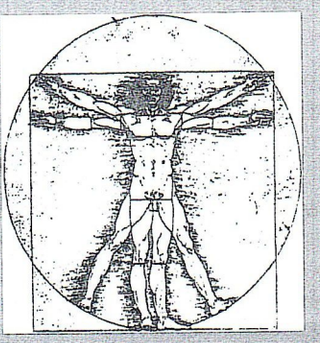

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MORPHOLOGY OF MICROGLIA IN CEREBRAL CONTUSIONS

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Abstract

Objectives: To present morphological changes of microglia depending on time passed from injury to death in persons with brain contusions.

Materials and methods: Samples were taken from nine cases with different survival time. Range of survival time was from under 1 hour to 144 hours. Samples were stained with ABC method of double staining for CD68 and Iba-1. Microglia were revealed and defined with Iba-1 staining. If microglia has black granulated staining besides the brown, that is shows immunoreactivity for CD-68, it will be differentiated as activated microglia.

Results: There were no significant changes in morphology of microglia in cases with short survival time and survival time under 12 hours. Significant changes in morphology of microglia were noticed in cases with longer survival period, between 96-144 hours.

Conclusions: We found significant differences in microglia morphology between the cases with short survival time and those with more than 4 days of survival time. This shows that brain tissue injury presents sufficient stimulus for activation of microglia and their transformation is time-dependent.

Introduction

Pío del Río Hortega [1] was the first scientist who performed microscopic analysis and presented the concept of microglia as cellular element in the central nervous system, in 1920. Hortega's concept of microglia consists of the following: 1 Microglia enter the brain in its early development; 2 This cells have amoeboid morphology and are of mesodermal origin; 3 Microglia use the blood vessels and the white matter pathways as guiding structures for migration and entrance in the brain; 4 Microglia are transforming into ramified cells known as quiescent or "resting" microglia in a mature brain; 5 In a developed brain they are almost equally allocated trough the entire CNS, with small variations; 6 Every cell has its own territory; 7 Upon disturbance or encountering specific brain pathology, microglia transforms and receives amoeboid morphology such as in the beginning of their development; 8 Microglia have the capacity to migrate, multiply and phagocyte.

Microglia constitutes 5-20% of the total number of glia cells in the brain [2]. Microglia have different morphology depending on the conditions in the CNS and the spinal cord. The morphology is determined and dependant on the function of the cells. When found in a normal physiological state, microglia are described as cells with small cellular body and numerous long branching processes. They are constantly patrolling the brain tissue, surveying the surrounding area for agents that would disturb the tissue homeostasis. This type of microglia is referred to as quiescent or "resting" microglia, even though they are in constant motion. The term "resting" indicates the phase in which they are ramified with small cellular body.

The most important function of microglia arises from their capacity for activation upon disturbance or encountering specific brain pathology. The activation of microglia that occurs when lesions of the CNS happens, is characterized by numerous events relating to the cellular morphology, cellular size, cellular counts, as well as the molecular changes which include the type of the expressed cell surface markers (immunophenotype) and the production of cytokines and growth factors [3].

The details concerning the microglial activation may vary depending on the type of injury; nevertheless a generalization of certain constant processes that occur can be made. The change of the cellular morphology is the one most frequently described. Once activated the cells undergo several key morphological changes including thickening and retraction of branches. With the cellular hypertrophy there is increased expression of the CD3 complement on the cellular surface [4]. Both cellular hypertrophy and the increased expression of the complement are manifested 24 hours after the injury. Microglia counts increases 2-3 days after the injury, with the maximal counts after the 4-7th day. The changes of the immunophenotype mostly occur with the increased expression of MHC antigens 4-7 days after the injury. It is important to note that the microglial activation that follows after acute cerebral injury presents transitory and controlled event with a usual duration of 1 month, even in the case of severe damage of the CNS [5-9]. The aim of this study is to show the morphological changes of microglia in correlation with the survival time in persons with brain contusions.

Materials and methods

To accomplish the aim of the study samples were taken from brain contusions from persons with different survival time: three cases with no more than 1 hour survival time, three cases with no more than 12 hours survival time, and three cases between 96-144 hours survival time.

Protocol for brain tissue fixation

The cerebrum, cerebellum and the brain stem are measured, and the weight is noted in grams. The surface of the brain is inspected for possible presence of contusion. The localization and dimension of each found contusion is being noted and photographed with a ruler. With the use of a macrotome, each of the cerebral hemispheres is cut into coronal sections with a 20 mm interval. The tissue sections are placed into "sandwiches" made of plastic perforated plates, sponge and filter paper. The brain tissue is fixed into 8 liters of 10% neutral buffered formalin at room temperature. After exactly five days, the fixed sections of brain tissue are being removed from the sandwiches, and put into at least 4 liters of phosphate buffer with 0.02% sodium azide at +4 °C degrees.

Protocol for obtaining brain tissue samples for preparation of microscopic analysis samples

Excision of the contusion is being made along with 1 cm of the tissue surrounding the contusion. The obtained samples are cut into a 3mm interval. The pieces are placed into histology cassettes and are being photographed. The tissue from the cassettes is embedded into paraffin blocks, and the paraffin blocks are being cut with a microtome with a 40-micron interval.

Immunohistochemical protocol

Immunohistochemical staining on a short time fixed human tissue with the use of ABC (Avidin Biotin Complex) method of double staining for CD-68 (KP 1, Dako) with Nikelsulphat and for Iba-1 (Vako) with DAB (Diaminobenzidine). Iba-1 is presented as brown coloration, and CD68 as black coloration. Microglia are revealed and defined with Iba-1 staining, if microglia has black granulated staining besides the brown, that is shows immunoreactivity for CD-68, and it will be differentiated as activated microglia.

Microscopic (imaging) analysis

The description of the morphological changes of identified microglia was performed on photography of the stained microscopic slides made on Leica 5000MB microscope using 60x oil lens and software Visiopharm.

Results

The study includes nine cases with different time of survival. The cases were divided into three groups: three cases with no more than 1 hour survival time, three cases with no more than 12 hours survival time, and three cases with survival time from 96 to 144 hours. All of the cases were male, with average age of 67 years. The average weight of the brain was 1325 grams. The postmortem period for all cases was less than 24 hours. All of the cases were injured in traffic accidents, and death was due to brain injury.

The cases with very short survival time showed well-expressed Iba 1 staining, which shows the structure and morphology of microglia. The cellular body is oval and slightly elongated and numerous long branching processes. This type of microglia is referred to as resting, that is, ramified microglia. Inside the cellular body and some of the processes you can see small black spherical stains due to the positive CD 68 staining indicative for presence of intracellular protein in the cellular lysosomes (Fig.1).

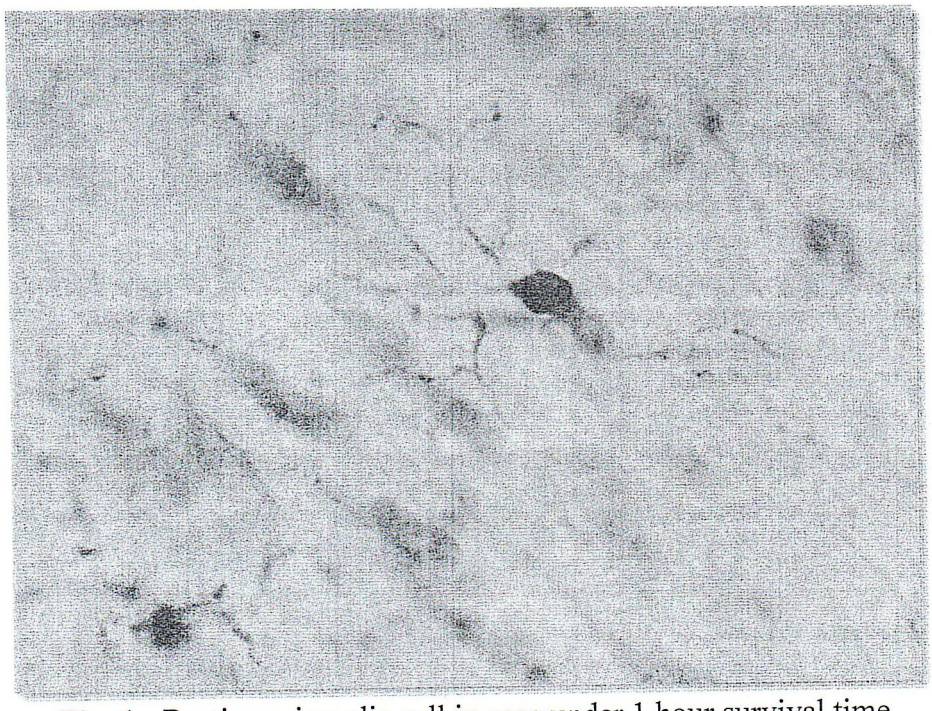

Fig. 1.- Resting microglia cell in case under 1 hour survival time

In the cases with no more than 12 hours survival time, morphological changes of the cell can be seen, although they are not drastic. The cellular body increases and elongates, and the processes are still long and branched out. The CD 68 positive staining is intensified but with a small difference compared to the intensity in the cases with very short survival time (Fig 2).

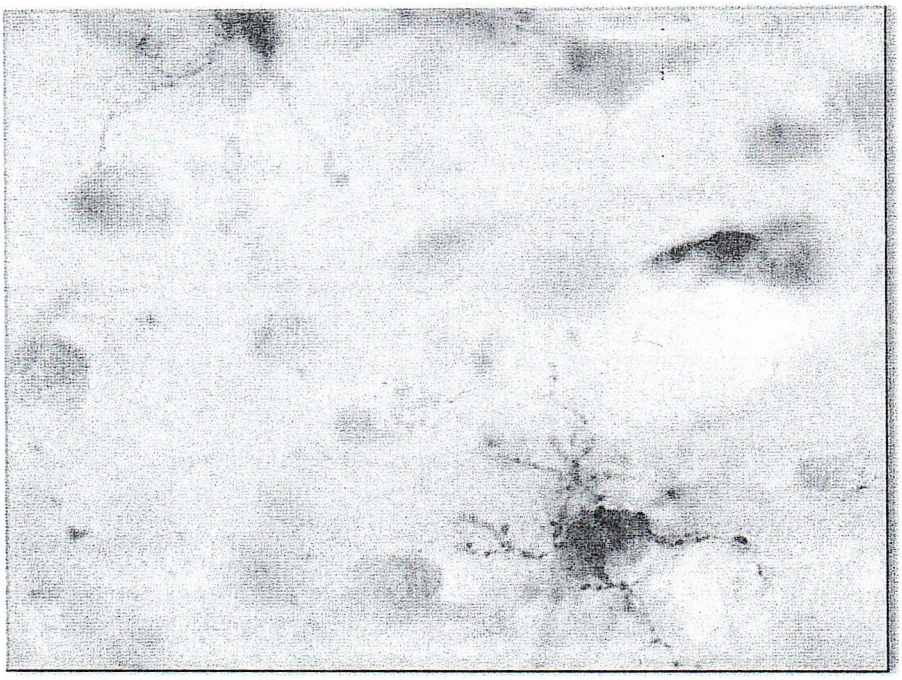

Fig. 2.- Resting microglia cell in case under 12 hours survival time

The microglia in the last group shows significant difference in morphology compared to the previous cases. The cellular body is amoeboid with very short processes which are almost invisible in places. In the fully activated cells the CD 68 staining (black) is dominant over the Iba-1 (Fig.3,4).

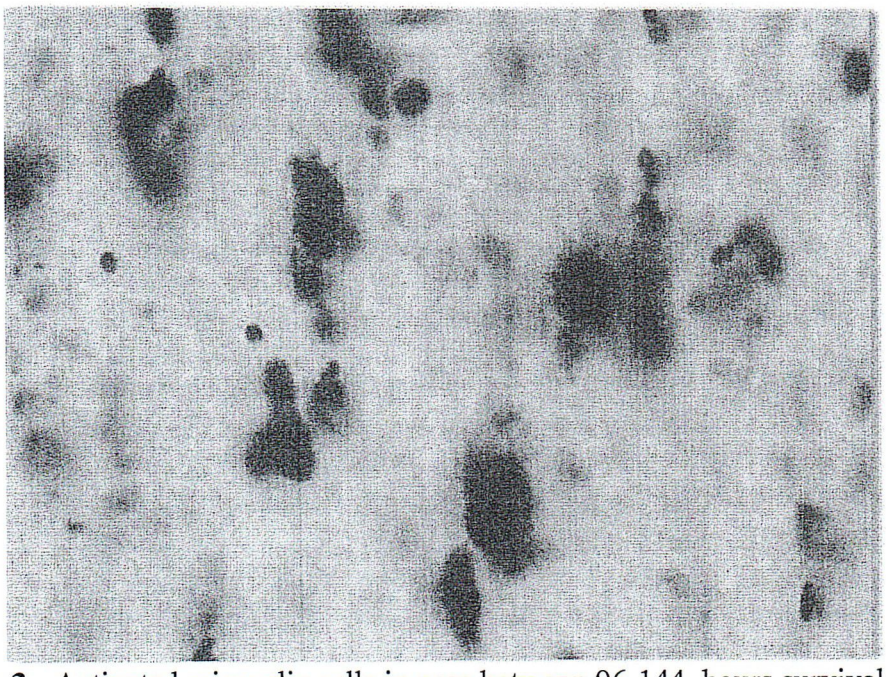

Fig. 3.- Activated microglia cells in case between 96-144 hours survival time

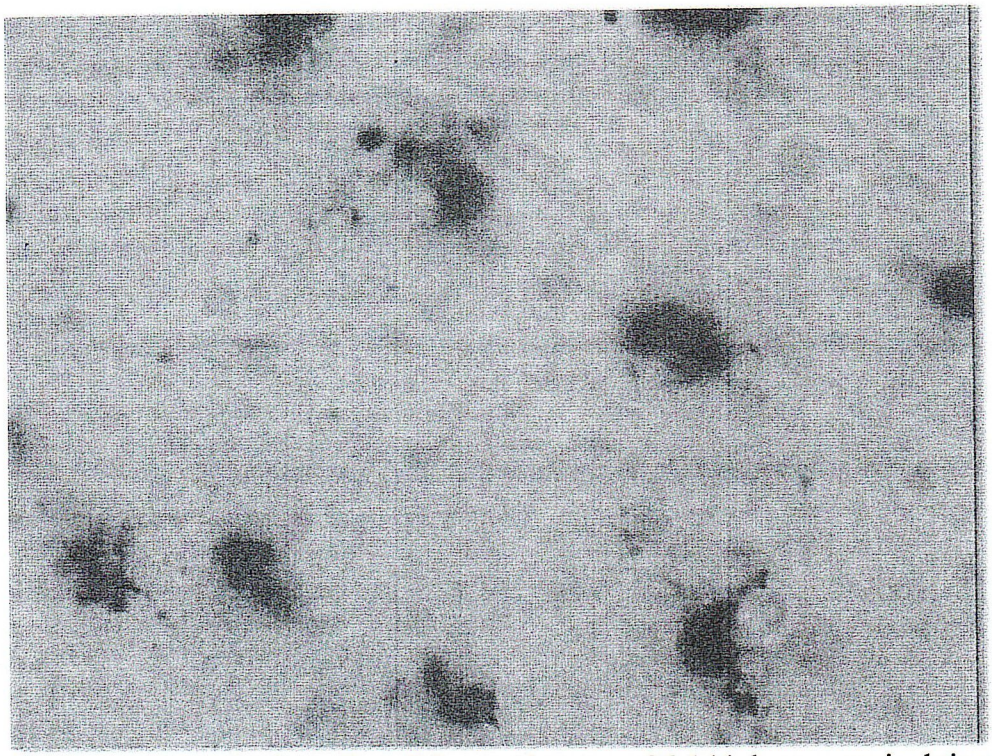

Fig. 4.- Activated microglia cell in case between 96-144 hours survival time

Discussion

Although considered as cerebral macrophages, at first glance, the morphology of microglia in a healthy, adult cerebral tissue is not indicative for any connection with the macrophage morphology. Based on their morphological appearance they got their name "ramified" microglia. In this state they have small cellular body and long branching processes. The term "resting" is used to explain the state they are in, that is to make connection between the morphology and the function of these cells. The morphological changes of microglia are dependent on the health condition of the central nervous system. Every change in the tissue homeostasis whether incurred by disease or trauma, sends a signal to the defense cells that react adequately with numerous morphological changes. Under the influence of the defense cells the microglia undergo morphological changes and transform from resting into activated microglia. These changes are characterized with reduction of the complexity of their form. The processes are shortened and from hyper ramified they transform into amoeboid with the formation of protrusions and increased motility [3,10-11].

In brain contusions the primary mechanism of injury is a result of the mechanical damage of the cerebral tissue and thus neurons, axons, glia cells and blood vessels. The mechanical damage is followed by the secondary mechanism of injury as a result of neuron damage. The secondary injury includes ischemia, lipid degradation, formation of free radicals, and release of protease leading to demyelination, axonal degeneration, neuron death which ends with glial scar formation [12-16].

Trauma of the CNS is a sufficient signal for microglial activation. Microglia have the leading role in the immune response as well as activation and recruitment of other defense cells. In our study we have presented nine cases with different survival time divided into three groups. It allowed us to monitor the cellular transformation in period from 30 minutes to 144 hours. The results confirmed the existing knowledge of morphology changes of microglia and their activation depending on the time frame.

Thus, in the cases with a very short survival time, the cellular morphology was not changed, that is survival time of 30 minutes is not sufficient for their activation and reaction. In the second group with up to 12 hours survival time (2,4, and 12 hours) a morphological change in microglia is noted.

The cellular body enlarges and the positive CD68 staining is indicative of lisosomal activity which leads to activation of the microglia. In the period between 96 and 144 hours (96,120, and 144 hours) the morphological changes and activation is drastic and visible compared to the other two groups. The branched form is lost, the processes are partially present and the cells transform into amoeboid form, which proves their similarity with macrophages. The published results indicate different periods of morphology change of microglia but in general peak activation is achieved the seventh day after the injury [17]. The microglial reaction to injury is very fast; 4 hours after the injury they increase their motility, they mobilize and direct towards the lesion. In IN VIVO imaging studies rapid proliferation towards the lesion was documented as a response to the extracellular ATP released from the injured tissue [18-20].

The earliest documented activation in human tissue is 72 hours after injury and it can maintain months after it [20]. This shows that microglia besides the destructive role, have also protective one and they actively take part in the process of tissue healing.

Conclusion

In this study we focused on the morphological changes of microglia in correlation with the survival time in persons with cerebral contusions. We found significant differences in their morphology between the cases with short survival time and the cases with more than 4 days of survival time. This shows that brain tissue injury presents sufficient stimulus for activation of microglia and their transformation is time-dependent. In further research we will focus on determination of the microglial counts on the place of the injury and opposite of the place of the injury, as well as, the impact of the time distance on the microglial counts.

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