UNIVERSITY OF MEDICINE AND PHARMACY

CRAIOVA

PhD STUDIES

PhD THESIS

ABSTRACT

MACROSCOPIC, MICROSCOPIC AND IMMUNOHISTOCHEMICAL ASPECTS OF ENCEPHALON IN TRAUMA PATIENTS EXAMINED BY FORENSIC MEDICINE

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CRAIOVA – 2015

Introduction

Traumatic brain injuries (TBI) are a major cause of death and invalidiY worldwidely and, as a consequence, they require prevention measures and permanent monitoring. TBI are non-degenerative, non-congenital lesions of the nervous central system, generated by an external mechanical force that may lead to a permanent or temporary damage of the physical, cognitive and psychosocial functions, asssociated with a diminishing or change of consciousness (Faul M, Coronado V, 2015; Zink BJ, 2001).

Traumatic brain injuries affect million of people all over the world. Numerous studies showed that traumatic brain injuries represent a public healthcare problem, both due to the fact that they are considered the main cause of violent death, and also because they are invalidity generators in survivors.

CHAPTER I. Nervous system ontogenesis

The nervous central system develops from the neural ectoderma that lies on both sides of the medial line, on the dorsal part of the embryonic disc (Nakatsu, et.al, 2000). The cells in these region are elongated, multiply a lot faster and, thus, in the third week of intra uterine life, there produces a thickness of the ectoderma, forming a multi-stratified structure called the neural plaque or blade, made up of a single layer of neuroepithelial cells. Various induction factors produced by the notochord cells determine the differentiation of this plaque, thus acquiring a pseudostratified epithelium (Saitsu H et al., 2004). The junction areas between the neural plaque and ectoderma are called neuroectodermal junctions, consequently leading to the development of neural apices.

The neural plaque starts to go deep into the subadjacent mesenchyme, its margins converge and, by their fusion on the medial line, they generate the neural tube, in the sixth week. The neural tube fusion begins in the embryos with 4-6 somites. The neural tube lumenus will give birth to the neural tube. On the lateral and posterior sides of the neural tube, there will lay out the neural apices. Both the neural tube and the neural apices will go deep into the subadjacent mesenchyme, while the ectoderma will rebuild on their surface.

After the formation of the neuronal tube, the rostral part of the brain presents three dilations placed retrocaudally, known as the primary anterior brain or the prosencephalon, mesencephalon and rhombencephalon. The limits between the three primary brain vesicles are limited by early-developed fibers, transversally oriented. Brain development takes place at the same time with the flexures, that emerge before the neural tube closure; two are ventrally concave and one is dorsally concave.

The structure of the neural tube progressively changes, both by elongation and by convergent extension (Bay și Caspary 2012). From the histological point of view, the early neural tube is composed of a pseudostratified neuroepithelium, where the apical surfaces of the cells meet the neural tube lumenus and their basal or external surfaces are limited by the mesenchyme and by the neural apices through a basal membrane that will turn into a pia mater. At first, every cell of the neural tube has a motionless primary apical cillium, having a configuration of 9 micro tubules and an intra flagellar transport system. The membrane of the primary cillium expresses ionic receptors and channels, and modulates signaling ways for the Sst, Wnt and PDGFα (Lee si Gleeson, 2010). The neuroepithelium contains stem cells that will give birth to various populations of neuroblasts and glioblasts.

The neural tube wall will rapidly develop, gradually reducing the neural channel diameter, therefore in the foruth week of intra uterine life, the neural tube presents various well-differentiated microscopic structures: the internal lining membrane, the ependimary region or proliferative region formed by apolar neuroblasts, the remaining cells will transform in ependimary cells when the multiplying processes cease, the middle region or the manta region where there migrate and differentiate the neuroblasts produced in the ependimary region that will subsequently become the gray matter, the marginal region made of the neuroblast prolongations in the subadjacent area that will generate upward and downward tracts and fascicles, subsequently developing the white matter and the external lining membrane (Mogoantă et al., 2004)

The processes of cellular multiplication and migration do not take place with the same intensity alongside the entire neural thickness. This phenomenon leads to the ventral and dorsal plaque emergence, made up of elements of the marginal region of two plaques simetrically situated in the posterior ½ of the neural tube, on both side of the medial plane. During the radial migration, the simple neuroblasts move from the ventricular surface to the pial one (Pleasure et al., 2000; Wilson et al., 2000; Nadarajah et al., 2002).

The cellular populations in the neuronal apices multiply and, together with the neurodilation occurring in the neural tube, they simultaneously progress to the rostral and caudal regions. The cells migrate from the apex to the brain neronal lines, before the tube closes. Neural apices will generate some elements of the peripheral nervous system (spinal and vegetative ganglions, Schwann cells, perineural staellite cells, satellite cells in the vegetative ganglions, nervous fibers). Also from the neural apices there will differentiate melanoblasts, odontoblasts, medula adrenal cells, C-cells in the thyroid and the APUD system cells.

CHAPTER II. Anatomy and histophysiology of the cerebral hemispheres

Brain hemispheres are the most developed segment of the CNS, constituting an organ with a wide structural and functional complexity. They are shaped as two semi ovoid masses, wider towards posterior than anterior and occupying over 80% of the skull cavity.

The telencephallus or the brain is divided into two brain hemispheres, left and right, separated by a deep trench called the inter hemispherical or longitudinal crack. In the basal part of this fissure, the hemispheres are bound together through a white mater thin plate called the corpus callosum. The external configuration of the large brain has an ovoid shape, with the long axis anteriorly-posteriorly oriented. Each hemisphere presents three extremities or poles, three sides and three edges. The extremities are: anterior (frontal pole); posterior (occipital pole) and lateral-inferior (temporal pole). The sides are: dorsal-lateral, placed in relation to the skull; medial side oriented towards the inter hemispheric fissure and basal, in contact with the base of the skull. The edges are: lateral, super medial and infer-medial.

The hemisphere sides present numerous grooves called or ridges. The deep grooves limit each cerebral lobe, while the superficial ones define the circumvolutions or gyrae. Thus, with a folded surface, the human brain is a “girencephallon” (brain with gyrus), in opposition to that of inferior animals who have a smooth surfaced brain, called “lisencephallon”.

The cerebrum consists of the gray and white matter. Gray matter is disposed on the surface to form the cerebral cortex, but it is also present at the base of the hemispheres, where it forms the basal nuclei or striatum corpus. The cerebral cortex is composed of neurons with different types, shapes and sizes, arranged in unevenly distributed multiple layers. In addition to the nerve cells, the cerebral crust contains nevrogliae, nerve fibers and blood vessels. Each cerebral hemipshere has one super lateral side, a medial and an inferior one (or basal), separated by the super medial, infer lateral, orbit median and occipital-medial margins,.

The super lateral side surface is convex and is situated under the cranial vault bones; the frontal, parietal and occipital lobes correspond roughly to the surface of these bones to which they their names are related. The frontal and parietal lobes are separated from the temporal lobe through the lateral groove (Silvius).

The inferior surface is divided by the anterior part of the lateral groove into a small orbital-anterior zone and a tentorial, larger zone, towards posterior. The orbital part is the concave orbital surface of the frontal lobe and it rests on the floor of the anterior cranial fossa. The posterior part is made up of the basal areas of the temporal and occipital lobes and rests on the downpart of the middle cranial fossa and the superior surface of the cerebral tentorium, which separates it from the superior cerebellum surface. The medial surface is flat and vertical, separated by the opposite hemisphere by the longitudinal fissure and the falx cerebri. Anteriorly, the brain hemisphere ends with the frontal and temporal poles, while posteriorly- with the occipital pole.

The cerebral grooves define the brain circumvolutions (gyrae), these being extensions of the subarachnoid space (Butler and Hodos 2005 Sarnat and Netsky 1981 Park et al 2007 Nishikuni and Ribas 2013 Catani and Thiebaut of Schotten 2012 Naidich et al 2013). When the grooves are anatomically constant and deep, they are called “fissures”. The main grooves have depths of 1 to 3 centimeters. The neuroarchitecture of the brain has been widely studied, and recent results have shown that there are regional differences of the cito-architecture of the cerebral cortex, which explains the physiological properties of these areas (Cavaglia M et al, 2001).

The white mater represents the most voluminous component of the cerebral hemispheres. Disposed under the cortex, white mater devoid of neurons, formed only of nerve fibers wrapped in myelin sheath, as well as nevroglial cells.

CHAPTER III. TBI - Clinical and epidemiological data

According to some studies, about 10 million cases of traumatic brain injury are recorded all over the world every year (J Ghajar, 2000; Brazinova A, Majdan M, et al, 2015). In the United States it is estimated that 1.7 million TBI occur anually, of which 1.3 million come to the Emergency Department, where approximately 80-92% of all cases are treated (Selassie AW, et al, 2013), requiring about 5.8 billion dollars as direct medical costs (Center for Disease Control and Prevention, 2014). TBIs represent about 40% of all deaths caused by acute injuries in the United States, whereas about 2% of the population lives with a disability caused by a TBI (MXL Faul, 2010).

Regarding the incidence of TBI, according to the latest figures from the Center for Disease Control and Prevention (CDC), in the United States the estimated average incidence of TBI between 2002 and 2006 was 576.8 for a population of 100,000.

Severe brain trauma mortality was estimated between 50-80%, especially in patients over the 60 years old. In Western countries, the results of administrative measures have reduced mortality from head trauma to 30-40% over the recent decades (Teasdale G, et al, 2012).

According to some statistical data, there are approximately 5.3 Americans living with a permanent disability due to severe brain trauma (D Thurman, Alverson C, et al, 1999). The

financial cost is estimated at around 4 billion dollars per year. This estimate includes patients with potential loss of income as well as that of the relatives of the patient (who could become caretakers), the cost of acute care, as well as other medical expenses, such as continuous rehabilitation care and ambulatory care.

CHAPTER IV. Clinical and statistical study of TBI

The clinical and statistical study of TBI was a retrospective one, performed by analyzing the forensic autopsy reports of the Forensic Medicine Institute of Craiova, between 2010 and 2014. Thus, the cases in which TBI was incriminated as the cause of death were recorded in the forensic reports to be the medical cause of death.

In this period, a number of 3260 autopsies were carried out to determine the medical cause of death in patients with trauma or presumed victims of trauma. Of the total of 3260 deaths, 622 (19.07%) were due to TBI.

According to gender distribution of TBI, it has been concluded that a number of 478 cases (representing 76.85%) occurred in men and only a total of 144 cases (approximately 23.15%) occurring in women, thus the male/female ratio was higher than 3/1. As of regarding the area of origin, it has been noted that in rural areas there had been 392 (63%) cases of TBI, while in the urban areas only 230 cases (37%), thus the ratio of rural/urban TBI was 1.7/1.

Following the age group distribution of TBI, it has been concluded that these may occur at any age, from newborns to the very old. In our study, we noted a significant, proportional increase of cases of death caused by TBI proportional to the increase of age. It was also noted that people over 50 years old were the most affected by severe TBI: from 622 cases of autopsied TBI, the number of people over 50 years old was 334, making up a rate of 53.70%.

The largest number of TBI cases (284 cases, 45.66%) was due to various accidents, which included all the death circumstances produced by TBI (e.g. falls, precipitation, animal attacks, falls from vehicles etc). In second place regarding the cause of death by TBI stood road traffic accidents with a total of 236 cases (37.94%). 27 cases (4.34%) of TBI were due to railway accidents. Overall, rail and road traffic accidents amounted to a total of 263 cases as cause of TBI, representing 42.28%.

Alcohol is one of the most important factors involved in the etiopathogeny of TBI. Assessing blood alcohol, 111 individuals with TBI had presented values between 0.2% and 3%.

Regarding the macroscopic aspect of meningeal lesions observed at the autopsy examination, 485 cases were found to contain several types of associated brain lesions (meningeal lesions, cerebral concussions and dilacerations, diffuse meningeal hemorrhage,

intra cerebral hematoma, intra ventricular hemorrhage etc); of these, there were 29 cases in which massive cerebral dilacerations were observed.

CHAPTER V. Histological and immunohistochemical study of brain changes in patients with severe TBI

The studied histological material was represented by brain fragments harvested while conducting the autopsy on a number of 35 deceased by TBI and forensically studied. The brain fragments were taken for histopathological diagnosis and for completion of the forensic diagnosis. For the histological study, hematoxylin-eosin staining and tricromic with Goldner-Szekely colorings were performed, while for the immunohistochemistry exam antibodies were used: anti-GFAP (clone 6F2, Dako) for highlighting the glial cell reactions; anti-CD34 (clone EP373Y Abcam) for microvascularisation highlighting and anti-CD68 (clone KP1, Dako) to highlight macrophage cell types.

The microscopic examination of brain tissue fragments collected at necropsy revealed a multitude of injuries depending on the severity of the lesional impactand on the time elapsed from the occurance of the injury to death. The most common injuries were meningeal hemorrhage and/or intraparenchimatous hemorrhage. The recent leptomeningeal hemorrhages were characterized by diffuse infiltration of the leptomeningium with varying amounts of blood, in which the red cells appeared to be intact, while for the case of older hemorrhages the presence of partially hemolyzed red blood cells and a large number of macrophages. Various sized hematoma were highlighted intraparenchimatous, recent or old, diffuse petechial hemorrhages or perivascular bleeding through the Virchow-Robin spaces.

The immunohistochemistry examinations showed that the changes of the cerebral microvascularization were highly variable depending on the type of brain injury, the patient's age, the time elapsed from the moment of aggression to death. Leptomeningeal recent or older hemorrhages had caused a substantial reduction of the microvascularization within the cerebral parenchyma, most likely through the increase of intracranial pressure. In a young patient (21 years old) with a brain injury older than seven days, we found an increased number of vessels of neoangiogenesis within the focus of intraparenchymatous hemorrhage. The macrophage-system cell reaction (microglia, blood macrophages) was very low in recent TBI, yet it was very intense in trauma that had occurred at more than one week prior to examination.

The assessment of the glial cells reaction allowed us to remark the fact that the intensity of these cells reaction depends on the age of the TBI, as well as on the time passed since the installation of the lesions until the persons' decease. In recent TBI, under 48 hours, the glial cell reaction was very low, while amidst the older lesions, especially in the case of younger patients, the glial cell reaction was very intense.

CHAPTER VI. Histological and immunohistochemical study of the healing process of brain injuries following penetrating TBI

In order to conduct the study, 15 common Wistar adult rats were selected, weighing between 270 and 310 grams, from the Biobase of the University of Medicine and Pharmacy of Craiova, upon which penetrating TBI were inflicted, by needle-stabbing at the level of the parietal lobe of the right cerebral hemisphere. Afterwards, the animals were divided into 3 groups of 5 animals and were sacrificed at 1, 3 and 7 days after the trauma had occurred. The brain fixing was performed in 10% formalin for 5 days, after which it was included in paraffin. For the histological study hematoxylin-eosin staining was used, whereas for the immunohistochemical study antibodies were used: Anti-Iba 1 (code ab5076, Abcam) for determining microglia and macrophage reactions; anti-GFAP (code M0761, Dako) for astrocyte highlighting; anti-CD34 (code Ab81289, Abcam) for the cerebral microvascular study; Anti-Neu N (code ab128886, Abcam) for highlighting neural changes.

The histological study showed that the trauma caused by us had destroyed an area of the parietal gray mater, the cortical white mater and the hippocampus, resulting in the production of a longitudinal cavity with irregular walls caused by the infliction of nerve tissue dilacerations. After 24 hours following trauma, within this cavity we observed an accumulation of large amounts of blood, especially in the deep part of the wound, as a result of destroying the cerebral vascular network. The neural suffering started to be obvious after 24 hours from the production of the lesion. Around the wound, the neurons presented a multitude of morphologic changes varying from cell necrosis processes, with the occurance of “neural ghosts”, nuclear condensation, cytoplasm vacuolation, to neural ischemia phenomena (red neurons).

The astrocytes within the lesion area were totally destroyed, while perilesionally they were partially altered. The macrophage system cells were the first to react following the traumatic aggression, after 24 hours from infliction. In our experiment, at the periphery of the lesion (perilesional) there had appeared numerous round cells, of elevated sizes, intensely reactive towards the antibody anti-Iba1. The analysis of the traumatic area had permitted us to remark the fact that the cells of the macrophage system which are present within the cerebral post-traumatic lesion come both from the activation of microglia present inside the cerebral tissue as well as from the monocytes in the blood. Three days after the TBI, the neural necrosis area was much more extensive, the reaction towards the antibody anti-NeuN being absent within large perilesional areas.The GFAP and Iba1 immunohistochemical reactions showed the presence of numerous, intensely reactive astrocytes as well as that of macrophage type cells, also perilesionally. In the perilesional area, by using the antibody anti-CD34 there had been identified numerous neo-angio-genesis capillaries, as well as isolated CD34-positive cells.

The microscopic study of the histology sections obtained 7 days after the infliction of the TBI has shown that the dimensions of the wound had reduced due to proliferation within the perilesional area of numerous monocyte cells. Also here, we have identified areas with small restant hemorrhagic suffusions and cells with hemosiderin pigment within their cytoplasm. Assessing the viability of neurons using the antibody anti-NeuN, it has been clear that they were destroyed over large areas. Within the cerebral parenchyma a dense, unorderly arranged cell population was identified, which had altered the normal cellular architecture of the cerebral cortex. Using the antibodies lba1 and GFAP, it was concluded that within both the wound area and also perilesionally there had appeared a particularly large number of macrophages and reactive astrocytes. Our study has shown that the healing processes of the cerebral tissue following a severe TBI is realized through the according participation of astrocytes, microglia/macrophages and of the endothelial cells, while concomitantly the neural damage extends over large areas.

CHAPTER VI. Conclusions

- Traumatic brain injuries have become an evergrowing public helathcare problem, in our study representing about 19% of death caused by trauma.
- The particularities of the studied group were the following:
  - the large number of deceased men (478 cases, representing 76.85%), compared to the number of women (144 cases, representing about 23.15%), men-women ratio being higher than 3/1. Similar studies in Europe and USA found a men-women ratio of maximum 1.7/1;
  - the high number of recorded TBIs in the rural area (392 cases, 63%), compared to the urban area (where there were recorded only 230 cases, representing 37%), the rural/urban ratio being 1.7/1. Most of the epidemiological studies found a higher number of serious TBI in the urban area caused by traffic accidents;
  - TBI distribtuion on age groups showed a significant, proprotional increase by age of the death cases by TBI; all epidemiological studies found a higher percentage of TBI in children under 10 years old and in the elderly over 70 years old;
  - alcohol intake represented a major risk factor. Of 252 investigated cases, 111 (representing 44%) had high levels of blood alcohol.
  - car and train accidents summed up a total no. of 263 cases, representing 42.28%.
- Most TBIs resulted in complex lesions; therefore, in 485 cases there were observed various types of associated brain lesions (meningeal lesions: concussions and brain dilacerations, diffuse meningeal haemorrhages, intracerebral hematomas, intraventricular blood looses).

- The histopathological and immunohistochemical examination highlighted the complexity of traumatic lesions in tissues and cells.
- The experimental histopathological study on animals showed the healing possibility of the penetrant brain lesions, limited by astrocyte participation, and also by the extension of neuronal destructions, thus explaining the complex neuropsychological symptoms or handicaps of persons surviving a TBI.

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