

UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

PH. D. THESIS

ABSTRACT

**STUDY OF INFLAMMATORY REACTION IN ISCHEMIC
STROKES PATIENTS**

PH.D. SUPERVISER

Prof. LAURENȚIU MOGOANTĂ, M.D. Ph.D.

PH.D. STUDENT

RALUCA MARIA PĂVĂLOIU, M.D.

CRAIOVA

2018

TABLE OF CONTENTS

A. GENERAL PART

Introduction	3
CHAPTER I	
Development of the central nervous system.....	3
CHAPTER II	
Vascularization of the central nervous system.....	4
CHAPTER III	
Histology of the encephal.....	5

B. PERSONAL CONTRIBUTIONS

CHAPTER IV	
Objectives.....	5
CHAPTER V	
Clinical and statistical study of the ischemic strokes between 2010-2014.....	6
CHAPTER VI	
Histological study of the ischemic strokes.....	7
CHAPTER VII	
Immunohistochemical study of the ischemic strokes.....	8
CHAPTER VIII	
Evaluation of inflammatory reaction and changes in cerebral parenchyma in rats treated with NSAIDs and neurotrophic.....	9
CONCLUSIONS	11
BIBLIOGRAPHY	11

Keywords: ischemic strokes, inflammatory reaction, immunohistochemistry, histology, anti-inflammatory and neuroprotective therapy.

A. GENERAL PART INTRODUCTION

Stroke is one of the most important public health problems with a strong socio-economic impact worldwide and is also one of the main causes of disability in highly developed countries.

Even though it is the third cause of death, after heart disease and various types of neoplasm, in terms of social costs and disability, stroke is the first.

Worldwide, strokes cause deaths annually, five million lives are estimated, and severe disabilities are affecting another five million people [1].

In Europe, the incidence of strokes varies from one country to another, with an estimate of between 100 and 200 new strokes per 100,000 people annually, this figure representing a huge burden for the economy.

Statistics show that the maximum incidence of stroke occurs in 75% of cases after age 65 [3,4], age associated with a more difficult recovery after vascular accident [5,6,7]. Mortality after stroke is today between 20 and 30%.

Patients who survive a stroke often have symptoms that include: motor function paralysis, sensory deficits, perceptual, balance deficits, speech disorders, depression, dementia or other cognitive impairment [5,8,9]. These data suggest that stroke is a real health problem, not only by the high rate of mortality but also in motor performance and cognitive consequences of survivors. Sequelae of stroke can affect the quality of life of the patient and his family due to the disabilities they cause.

CHAPTER I DEVELOPMENT OF THE CENTRAL NERVOUS SYSTEM

The nervous system is the most complex physical system known to the human species that ensures the integrity and coordination of the internal organs functions.

The nervous system appears higher form neurological management, consisting of billions of interactive units.

Nervous tissue enters in the structure of the central nervous system (spinal and encephalic) and the peripheral nervous system (spinal nerve and spinal cord).

The neural ectoderm cells will differentiate in two directions: primitive nerve cells (neuroblasts) and supportive cells (spongioblasts). Neuroblasts are shunky cells for all types of neurons. Spongioblasts will generate macroglia, while the microglia will form from blood mononuclear cells [20].

The mature nervous system contains a wide range of cell types, which can be divided into two main categories: neurons, primarily responsible for signaling and supporting cells, called glial cells.

CHAPTER II VASCULARIZATION OF THE CENTRAL NERVOUS SYSTEM

Although the brain is only 2% of the body weight, it receives approximately 20% of the cardiac output, and normal brain functions can only be achieved under adequate cerebral blood flow, depending on the physiological intake of oxygen and nutrients.

Total cerebral blood flow is approximately 750 ml / min. This flow is provided by the two carotid arteries and basilar artery, each contributing approximately 250 ml / min. The total intracranial blood volume is 100-150 ml at any time; thus the circulating intracranial volume performs 5-7 cycles per minute [54].

Brain vascularization comes from two major sources: the carotid system and the vertebrobasilar system.

The carotid system is responsible for the vascularization of the anterior 3/5 of the encephalon (the entire frontal lobes, parietals and partial temporal lobes), and the vertebral-basilar to the 2/5 posterior vasculature (deep vein lobes, occipital lobes, cerebral trunk and cerebellum).

The carotid and vertebral arteries are located in the subarachnoid space, and their branches form an anastomotic system at the lower surface of the encephalus. This anastomotic system is a functional reserve, protecting brain tissue in situations where occlusions or stenoses of cerebral vessels occur [53].

CHAPTER III HISTOLOGY OF THE ENCEPHAL

The nervous system is made up of over 100 billion neurons that ensure the body's

integration into the external environment and coordination of internal organ functions.

One of the most complex systems in the human body, the nervous system is made up of an organ assembly consisting of nervous tissue, blood vessels and connective tissue. The vascular elements are separated by the nervous tissue through the blood-brain barrier, a selective permeability structure.

Macroscopically, the CNS consists of the following components: gray matter, white matter and cross-linking [90].

The nervous system consists of parenchyma and stroma. The parenchyma of the central nervous system consists of the totality of nerve cells. The tree is made up of glial cells, fine connective elements and blood capillaries [91].

The gray substance is in the form of spines (spinal cord), islets (inside the cerebral trunk and diencephalon) or is expressed in a continuous layer (at the surface of cerebral and cerebral hemispheres).

The white substance is mainly formed from parallel myelinated axons, grouped in bundles and cords.

The crosslinked formation is composed of a mixture of gray matter and white matter, without being well defined, and consists of neurons with very branched dendrites and many synapses.

B. PERSONAL CONTRIBUTIONS

CHAPTER IV

OBJECTIVES

In this thesis we intend to perform:

- 1. A retrospective clinical-statistical study** of ischemic stroke at a university clinic that highlights the following aspects of the condition: establishing the incidence of ischemic stroke incidence on a four year period, distributed by sex, background and groups age; evaluating the modifiable vascular risk factors; the evolution and prognosis of ischemic stroke with the specification of neurological factors and comorbidities that lead to unfavorable developments; specifying the role of early brain imaging exploration in the emergency diagnosis and the evolution of acute ischemic stroke; exposure of major complications that accompany strokes.

2. A histological study of cerebral tissue derived from ischemic stroke deaths, but also on parts from adult rats treated with neurotrophic and anti-inflammatory drugs to highlight: the process of neuronal ischemia (the appearance of red neurons); changes in brain parenchyma at lesion level and perilesional; highlighting changes in brain microcirculation; the presence of the inflammatory process; highlighting the process of neuronal death by the occurrence of neural changes prior to neuronal death, neuronal ghosts.

3. An immunohistochemical study, completing the histopathological study to highlight: vascular changes; the viability of neurons; presence of inflammatory reaction; oligodendroglisis reaction; the pathway of apoptosis; the monocito-macrophage system; macrogly and microglia reaction; vascular regeneration.

CHAPTER V

CLINICAL STATISTICAL STUDY OF ISCHEMIC STROKES BETWEEN 2010-2014

We conducted a **clinical - statistical** epidemiological retrospective **study** on a 5-year interval (2010-2014), taking into account all the cases of ischemic stroke (6931) hospitalized in the Clinical Hospital of Neuropsychiatry Craiova. Both clinical observation charts and statistical data provided by the Bureau of Statistics of the Hospital were analyzed.

In regards to ischemic stroke distribution by sex, it was noticed that cerebral infarctions occurred more frequently in men - 51% and less frequently among women - 49%.

The study of cerebral ischemia distribution by background allowed us to observe that there are major differences between urban and rural areas - 61% of cases originated from rural areas and only 39% from urban areas, raising special issues regarding rural healthcare, diet and preventive measures.

As to the distribution by age groups, the highest incidence was recorded in the interval 65 - 74 years - 33%. Annual distribution of ischemic stroke was very close from one year to the next.

Mortality in patients hospitalized with ischemic stroke during 2010-2014 in the Clinical Hospital of Neuropsychiatry Craiova, was of 3.3%, the lowest mortality being

recorded in 2013 of only 0,28% of all patients admitted with ischemic stroke and the highest mortality being recorded 2010 when the rate of death reached 0,95%. The mortality level recorded at the Clinical Hospital of Neuropsychiatry Craiova is extremely low, which does not correspond to the severity of the disease, as most stroke patients with unfavorable prognosis are discharged at the request of the family and their death is recorded at the patient's home.

Of the etiopathogenic factors analyzed, ischemic stroke is correlated in a very high percentage with hypertension (73%), ischemic cardiopathy (62%), smoking (38%), dyslipidemia (35%), atrial fibrillation (27,2%), alcohol abuse (26,8%), diabetes mellitus (16,8%), history of TIA (11,4%), obesity (4,8%).

Prevalent neurological dysfunctions on admission were motor dysfunctions - 52,5%, language and speech disorders - 31,1%, sensory - 4,8%, disorders suggesting damage to the posterior area - 6,5%, field of vision - 2,2%, impaired state of consciousness was recorded at admission in a relatively small number of patients (2,3%), which is explainable by the relatively rapid emergency presentation and hospitalization.

Basic brain imaging, both during emergency and hospitalization, was the computed tomography, conducted on all 6931 patients. As to the vascular topography of tomographic lesions, the most common were within MCA territory - 88,5% of all cerebral infarctions.

The main unfavorable prognostic factors in the studied group: age over 75 years and major comorbidities (diabetes mellitus, uncontrolled hypertension); "strategic" localizations of stroke and coma at onset; major neurological complications of ischemic stroke (hemorrhagic transformation, mass effect, and recurrence of stroke); cardiac complications (acute coronary syndrome and heart failure).

CHAPTER VI

HISTOLOGICAL STUDY OF THE ISCHEMIC STROKES

For the histopathological study brain fragments recolted during necropsy on 43 patients were used, clinically and imagistically diagnosed with ischemic stroke, admitted to the Neuropsychiatry Hospital of Craiova between 2010 and 2014. Histological dishes were colored with hematoxilin - eosin, the most used method of tissue staining.

Starting from the idea that the modifications of nervous tissue are extremely different from one area to the next, depending on the size of the ischemic focus, the vessels

affected by the ischemic physiopathological process, collateral circulation and possible anastomosis, as well as the general status of the cerebral vascular system, possible comorbidities, we evaluated the process of neuronal ischemia, changes in cerebral parenchyma in the lesion and perilesional (in the ischemic penumbra area), highlighting changes in cerebral microcirculation, the presence of the inflammatory process, highlighting the neuronal death process by the occurrence of neuronal changes prior to neuronal death.

It has been shown that in the lesion area the vast majority of neurons and glial cells have suffered a necrosis process. In areas at the periphery infarction site (ischemic penumbra), were revealed the presence of neurons characterized by "red neurons" or ischemic neurons, neuronal ghosts and condensed neurons. Inflammatory infiltration was moderate, with lymphocytes, plasma cells, granulocytes and rare macrophages. Cerebral ischemia has led to massive cell necrosis that has attracted leukocytes from the blood vessels that are indistinguishable, which suggests that inflammatory infiltration was better represented perivascularly.

In terms of cerebral parenchymal lesions, tissue necrosis with the disappearance of neuronal bodies, but also the spongy appearance of the cerebral cortex due to the degeneration of the nerve extensions; red neurons hypertrophied with the acidophilic cytoplasm as a result of the paralysis of mechanisms that maintain neuronal membrane homeostasis.

In the meningo-cerebrovascular vessels the most important changes were those of arteriosclerosis and vascular hyalinase with intraparietal lesions, which led to the disorganization of the arterial wall with various aspects: uniformly or irregular thick wall with many collagen fibers concentrically arranged in the middle tunic, with nearly total disappearance of smooth muscle fibers in the vascular wall structure or distorted wall with telescoping appearance.

CHAPTER VII

IMMUNOHISTOCHEMICAL STUDY OF THE ISCHEMIC STROKES

In addition to microscopy data, we also performed an immunohistochemical study, using the following immunomarkers: CD34 - used for highlighting vascular changes; NeuN

- to highlight neural changes; Olig2 - to see the oligodendroglial reaction; Caspase-3 - for the study of neuronal apoptosis; CD3 - for highlighting T lymphocytes; CD20 - for highlighting B lymphocytes; CD68 - highlights the monocito-macrophage system; IBA1 - to highlight microglia; GFAP - to highlight astrocytes.

Vascular lesions occurred in particular in the superficial part of the cerebral cortex, especially in the sub-surface vessels, their walls appear damaged and frequently with large perivascular edema and even with bleeding infiltrations, we also encountered a neural depletion, the deformation of the neuronal bodies and the condensation of the material nuclear, virtually in this area very few neurons were reactive to NeuN. Oligodendrocytes were equally susceptible to ischemia as well as neurons, revealing a negative reaction in the ischemic area.

To evaluate the process of neuronal apoptosis, we chose the brain areas near the ischemic outbreak to study. Although the process of cell death by necrosis, autophagy or autolysis is present in areas adjacent to the ischemic outbreak, the neurons also died by apoptosis present in perilesional areas and evidenced by the presence of a caspase-3 positive reaction.

At both the necrotic focal point and the rest of the cerebral parenchyma, the macrophage response was very intense.

Characteristic elements in assessing the immune response we have highlighted were the presence of infiltrated cells, predominantly T cells, mainly perivascular spread, while B lymphocytes were reduced, demonstrating the reactivity of each individual.

Glial cells, respectively microglia, quickly responded to cerebral ischemia, with the appearance of a large number of microglial cells at the necrosis site, and astrocytes reacted more in the ischemic penumbra area.

CHAPTER VIII

EVALUATION OF INFLAMMATORY REACTION AND CHANGES IN THE CEREBRAL PARENCHYMA IN RATS TREATED WITH NSAIDS AND NEUROTROPHIC

Experiments performed in this study were conducted during January 2016- February 2018 in the Biobase of the University of Medicine and Pharmacy of Craiova , on white Wistar rats aged 3 to 6 months. The experimental model of cerebral infarction was

achieved by cauterisation of MCA.

Rats were divided into three large study groups, each group having a total of 8 rats and the control group having a number of 4 rats as follows: the first group treated with NSAID, the second neurotrophic treated group, the third group treated with NSAID + neurotrophic and fourth group receiving physiological saline. The subgroups had a survival period up to the encephalus recolted of: 7,14,21 and 28 days.

As in the case of human encephal and on the rat recolted, we performed a histological and immunohistochemical study to determine the impact of neuroprotection agents on cerebral parenchyma after ischemia.

The histological study conducted in both the treated and control rats showed similar changes to those produced on human material, with the indication that as time progresses, the inflammatory response decreases in intensity, accentuates neuronal loss associated with various vascular changes, from vascular congestion to perivascular edema. Another finding was that the rate of brain tissue degradation slowed slightly within one month of producing ischemia over the severe degradation seen at the preceding time intervals, indicating that the remaining neurons (viable) began to adapt to conditions of cerebral hypoxia.

For a more accurate understanding of cerebral changes after ischemia, we also performed an immunohistochemical study, markers used for this time were: NeuN - for neural viability, GFAP - for astrocyte reaction, Caspase-3 - for neuronal apoptosis and alpha SMA - for myoepithelial cells in the vascular wall structure.

In the case of the untreated control group with NSAIDs and neurotrophic, the number of positive NeuN neurons was much lower. At the necrosis focal point, the neurons were completely destroyed, and the ischemic penumbra area had a greater stretch compared to those treated. In the control group, where the ischemic area has expanded considerably, the astrocyte reaction was absent, and in the ischemic penumbra area a moderate reaction was noted.

The areas chosen to study the neuronal apoptosis process were near the ischemic outbreak in the ischemic penumbra area because ischemic necrosis resulted in neuronal death including neurons that were in different stages of apoptosis, the results in the focal area being caspase-negative. The apoptosis process affected to the same measure both groups, being unaffected by the action of the molecules used.

CONCLUSIONS

Corroborating the results of the four studies, the following clinical and histological correlations were established:

- inflammatory reaction and cerebral tissue degradation were intense in the acute phase, decreasing in intensity as time progressed.

- the anti-inflammatory and neurotrophic agents used had the effect of limiting the ischemic penumbra area;

- all cerebral microcirculation showed microscopic changes in ischemic stroke, indifferent of the time elapsed from the lesion to the histological examination;

- major neurological complications of ischemic stroke - hemorrhagic transformation phenomenon, cerebral edema (histologically surprised in the form of perivascular and perineuronal edema) were microscopically detected, regardless of the time elapsed from the lesion to the histological examination.

BIBLIOGRAFIE

1. **Lopez AD**, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *Lancet* 2006; 367: 1747-1757.
2. **Rothwell PM**, Giles MF, Flossman E. et al. A sample scor to identify individuals at high early risk of stroke after a trancient ischemic attack. *Lancet* 2005 Jul 2-8; 366(9479):29-36..
3. **Barnett HJ**. Stroke prevention in the elderly. *Clin Exp Hypertens*. 2002 Oct-Nov; 24(7-8):563-71 .
4. **Brown AW**, Marlowe KJ, Bjelke B. Age effect on motor recovery in a postacute animal stroke model. *Neurobiol Aging*. 2003 Jul-Aug; 24(4):607-14.
5. **Badan I**, Platt D, Kessler C. et al. Temporal dynamics of degenerative and regenerative events associated with cerebral ischemia in aged rats.*Gerontology*,.2003 Nov-Dec; 49(6):356-65
6. **Markus TM**, Tsai SY, Bollnow MR et al. Recovery and brain reorganization after stroke in adult and aged rats, *Ann Neurol* 2005 Dec; 58(6):950-3.

7. **Asplund K**, Stegmayr B, Peltonen M. From the twentieth to the twenty-first century: A public health perspective on stroke. In: Ginsberg MD, Bogousslavsky J. (eds.) Cerebrovascular Disease: Pathophysiology, Diagnosis, and Management. Blackwell Science, Malden, MA, 1998: 901-918;
8. **Zhu L**, Fratiglioni L, Guo Z, Agüero-Torres H, Winblad B, Viitanen M. Association of stroke with dementia, cognitive impairment, and functional disability in the very old: a population-based study. Stroke, 1998 Oct; 29(10):2094-9.
9. **Craitoiu S**. Histologie – Curs pentru studenti anul II, Editura Sitech, 1999
10. **Choi BH**. Radial glia of developing human fetal spinal cord: Golgi, immunohistochemical and electron microscopic study, Brain Res Dev Brain Res 1981,1:249–267.
11. **Bajenaru O, Popescu O. Bogdan**. Elemente esentiale de neurologie clinica, Editura Medicala Amaltea, 2009.
12. **Hopkins J et al**. Human Biology and Health, Prentice Hall, 1993, 132–144.
13. **Hickey WF**. Basic principles of immunological surveillance of normal central nervous system. Glia 2001, 36: 118.