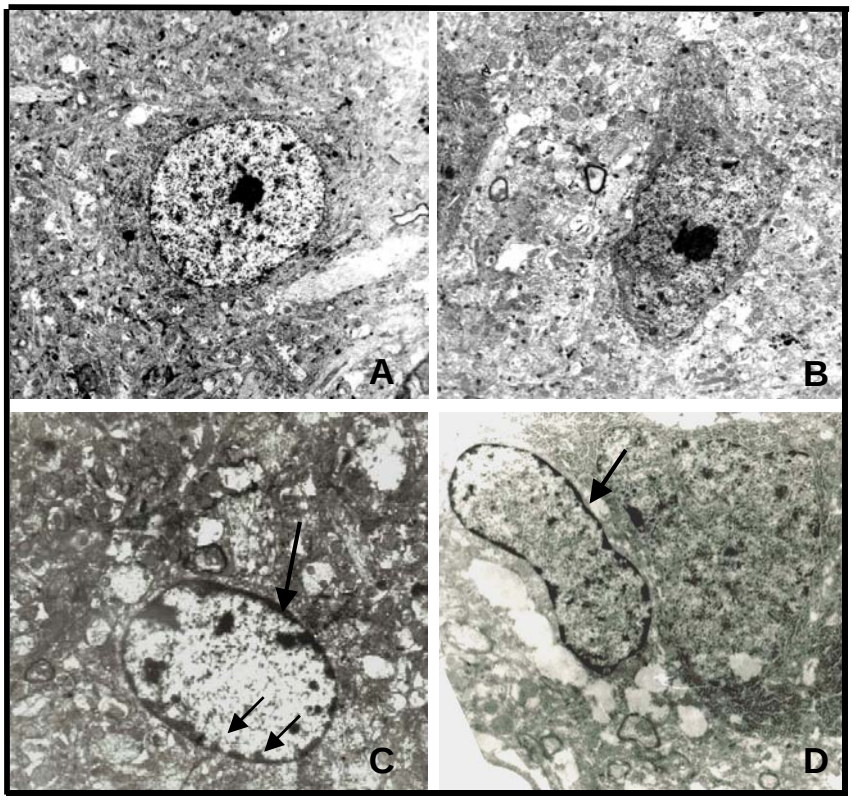
CELLULAR ALTERATIONS INDUCED AT AN EARLY TIME IN AN EXPERIMENTALLY BRAIN ISCHEMIA RAT MODEL.

Alma Ortiz-Plata[1,2], Carlos Sandoval[1,2], Daniel Rembao[1], Juan Nader[1], Fidel de la Cruz Hernández[2].
(1) Neuropathology Department. Instituto Nacional de Neurología y Neurocirugía, México D. F. (2)Experimental Pathology Department. Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional. México D. F. México. E-mail: aortiz@innn.edu.mx

Brain ischemia belongs to cerebrovascular diseases, which has an incidence of 80 to 85%. Ischemic stroke is a leading cause of death and disability in developed countries. Ischemia induce cell injure which is not the same for all cells, there is a selective neuronal death due to selective vulnerability that depends on neuronal density, different regional perfusion, and the intensity of the ischemia. The appearance of morphological ischemic alterations that could be easy observed as chromatin condensations takes some time [1,2]. In preliminary studies at 12 and 15 min of brain ischemia without reperfusion, DNA fragmentation was demonstrated by TUNEL in hippocampus and occipital region and by LM-PCR analysis in hippocampus region. The aim of this work was to evaluate the brain ischemia cellular injure at an ultrastructural level. Focal cerebral ischemia of the left hemisphere was produced during 12 and 15 min by the left internal carotid artery occlusion in Wistar rats. The animals were perfused with 2.5% glutaraldehyde - 4% paraformaldehyde, in PBS. Samples of the frontal, parietal, occipital, and hippocampal zone was dissected, OsO_4 postfixed, ethanol dehydrated, and epon embedded. One μm sections were toluidine blue stained; untrathin sections were uranyl acetate/lead citrate contrasted. Results: In previous work we analyze paraffin embedded and hematoxylin-eosin stained sections of the different brain zones, with 12 and 15 min of focal brain ischemia. We observed cellular groups that demonstrated nuclear pyknosis and excitotoxic edema, and in other places there were preserved cells of normal aspect with eosinophilic bodies, without edema, and little changes in the cytoplasmic volume and nuclear membrane. In hippocampus and occipital region there were also regions with individual cells without edema, obvious eosinophilia, well preserved nuclear and cytoplasmic membranes. Semithin sections of hippocampus and occipital regions show slight chromatin condensations near of nuclear membrane. Ultrastructural analysis show normal cells in control animals, with homogeneous fine dispersed chromatin. Cells with 12 and 15 min of ischemia showed little chromatin condensations join to nuclear membrane. Mitochondria and RE show preserved structure. Typically it was consider that ischemia produce necrosis injure, however there are reports that suggest the presence of apoptotic cell death [3], and in animal models it was observed cellular apoptosis after brain ischemia injure [4]. Also in these models it has been found structural and molecular characteristics that allow identifying the activation of apoptotic and antiapoptotic processes, which take place after reperfusion (blood circulation reestablishment). The morphologic alterations that were observed in this work at the initial times after ischemia, don't demonstrate the typical ultrastructural apoptotic characteristics, but in other hand, it has been suggested that the cell morphology after global transitory ischemia is not the typical one, and it could argue that the delayed cell death is different than the classical necrosis and apoptosis [5]. In addition, it has been reported that some cells, like neurons and chondrocites, displayed some characteristics that differ from the classical apoptosis, which suggest the existence of more than one pathway for programmed cell death reported by some authors as a neuronal paraptosis and chondroptosis respectively [6]. The typical morphologic evidences of apoptosis in human brain ischemia events are not considered a continuous characteristic. In penumbra zone (peripheral infarct zone) it can be found some death neurons in which chromatin condensation is a prominent characteristic that take place at early time, however the apoptotic bodies formation are unusual. The observations reported in this work show the initial morphologic changes induced by ischemia, which must been correlated with the molecules involucrate in this process, with the aim to continue the characterization of the alterations that are produced during cerebral ischemia in all cell brain types.

Referencias
[1] Castillo J. Rev Neurol 30 (5): 459-464, 2000.
[2] Mohr J.P., Martí-Vilalta J.L. Lacunes. En Barnett H.J.M., Mohr J.P., Stein B.M., Yatsu F.M., eds. Stroke Pathophysiology, diagnosis, and management. New York: Churchill Livingston; p. 599-622, 1998.
[3] MacManus J.P., et al. Neuroreport 5:493-496, 1994.
[4] Sugawara T. et al. J. Neurosci. 22:209-217, 2003.
[5] Ferrer I. Mecanismos de muerte neuronal en la isquemia cerebral. Rev Neurol 29:515-521, 1999.
[6] González, E.C., Kouri J.B. Microscopy Res Tech 64:269-278, 2004.



Control rat brain sections of occipital (A) and hipocamppus region (B), which shows normal morphology. Occipital (C) and hippocampus region with 12 minutes of focal ischemia. It can be observed slight chromatin condensation in little clusters join to nuclear membrane (arrows). Uranyl acetate/lead citrate 4,500 X.