THE THESIS OF MITOCHONDRIA AS MARKER OF LETHAL INJURY IN THE TRAUMATIC HUMAN BRAIN EDEMA. AN ELECTRON MICROSCOPIC STUDY USING CORTICAL BIOPSIES

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Received: November 1st,, 2007. Accepted: May 15th, 2008
Published on-line: May --, 2008

ABSTRACT
Cerebral cortical biopsies of 10 patients with clinical diagnosis of severe and complicated human brain trauma with subdural and extradural hematoma or hygroma were studied to establish mitochondrial morphological alterations and their relationship with nerve cell death. Cortical biopsies obtained in the surgical room were immediately processed by conventional technique for transmission electron microscopy. Three injured mitochondrial morphological patterns: swollen clear, swollen dense and dark degenerated mitochondria were found in neurons, degenerated myelinated axons, swollen and varicose dendritic process, and degenerated synaptic contacts. Swollen reactive astrocytes and hydropic oligodendroglial cells displayed clear edematous mitochondria. Isolated edematous mitochondria were embedded in the electron dense hematogenous edematous fluid localized in the enlarged extracellular space. At the level of open or collapsed cortical capillaries, edematous clear mitochondria were observed in endothelial cell and pericytes. Dark ischemic nerve cell, apoptotic neurons, and astrocyte, and ischemic hydropic oligodendrocytes were frequently found. Swollen clear, swollen dense and dark degenerated mitochondria were observed. The swollen clear mitochondria exhibited low electron dense mitochondrial matrix, and discontinuities of outer and inner mitochondrial membranes. Swollen dense mitochondria showed high electron dense matrix and swollen intact or fragmented cristae. Dense degenerated mitochondria displayed overall high electron density of matrix and mitochondrial membranes and cristólisis. The injured mitochondrial patterns are related with nerve cell death and postulated as markers of lethal nerve cell injury.

Key-words: Mitochondrial Damage, Traumatic Brain edema, Electron Microscopy.

RESUMEN
Se examinaron 10 biopsias corticales de pacientes con traumas severos complicados con hematomas o higromas subdurales, extradurales para establecer las alteraciones morfológicas mitocondriales y su relación con la muerte celular neuronal y neuroglial. Las biopsias corticales fueron obtenidas en el acto operatorio y procesadas por las técnicas convencionales para microscopía electrónica de transmisión. Se distinguieron tres tipos estructurales de mitocondrias dañadas: mitocondrias claras edematosas, mitocondrias densas edematosas y mitocondrias densas degeneradas en neuronas no piramidales edematosas, axones mielínicos degenerados, dendritas varicosas edematosas y contactos sinápticos degenerados. Los astrocitos reactivos y la oligodendroglia hidrópica exhibieron mitocondrias claras edematosas. Se observaron mitocondrias claras edematosas aisladas flotando en el fluído edematoso electrón denso presente en el espacio extracelular dilatado. A nivel de capilares corticales abiertos o colapsados se distinguieron mitocondrias claras edematosa en las células endoteliales y en los pericitos hinchados. Se encontraron células nerviosas isquémicas oscuras, astrocitos y células nerviosas apoptóticas y oligodendroglía isquémica densa. Las mitocondrias edematosas claras mostraron la matriz edematosa y electrón lúcida, discontinuidades de las membrana mitocondrial interna y externa y fragmentación de las crestas. Las mitocondrias densas edematosas exhibieron matriz oscura, cristólisis y fragmentación de las membranas mitocondriales. Las mitocondria densas degeneradas mostraron matriz oscura, y fragmentación de las crestas y de las membranas mitocondriales. Se relacionaron los patrones estructurales mitocondriales dañados con la muerte neuronal y glial isquémica y apoptótica. Se postulan las mitocondrias dañadas como marcadores de injuria letal.

INTRODUCTION

Functional, biochemical and fine structural studies of mitochondria and the respiratory system in experimental cerebral edema and brain ischemia and anoxia have been widely reported [1-23]. Different structural patterns and functional damage of mitochondria has been also previously reported in experimental and human traumatic brain injuries by photon correlation spectroscopy [24,25,], transmission electron microscopy [26,27],

biochemical and molecular studies [28], and proton magnetic resonance spectroscopy [29].

The goal of the present paper is to describe, by means of transmission electron microscopy and using cortical biopsies taken during neurosurgical treatment., the mitochondrial alterations induced by complicated traumatic human brain injuries associated to subdural and extradural hematoma or hygroma in the soma, myelinated axons, dendrites and synaptic endings of cortical non-pyramidal nerve cells of different cortical regions, in the soma and processes of astrocytic glial and oligodendroglial cells, and at the level of capillary wall. As a step toward characterizing mitochondria as lethal markers of nerve cell death, this communication try to establish the mitochondrial alterations following severe human brain trauma associated to extradural or subdural hematomas or hygromas, and the associated brain ischemia. To the best of our knowledge such studies has not been carried out until now.

MATERIAL AND METHODS

Samples of cerebral cortex of 10 patients with traumatic complicated head injury were used in the present study. Cortical biopsy was performed according to the basic principles of the Helsinki Declaration. Clinical data, diagnosis, biopsy region and degree of brain edema appear listed in Table I.

According with the intensity of traumatic injury, clinical symptoms and macroscopic observations in the neurosurgical room, the patients were classified into moderate and severe brain edema.

Two to five mm thick cortical biopsies were immediately fixed at the surgical room in 4% glutaraldehyde-0.1M phosphate or cacodylate buffer, pH 7.4 at 4°C. Later, they were divided into 1mm fragments and immersed in a fresh, similar solution for periods varying from 2-72h, followed by secondary fixation in 1% osmium tetroxide-0.1M phosphate buffer, pH 7.4 for 1h. They were then rinsed 5 to 10 min in a buffer, similar to that used in the fixative solution, dehydrated in increasing concentrations of ethanol and embedded in Araldite or embedded in Epon. For light microscopy thick sections of approximately 0.1 to 1µ were stained with toluidine blue and examined with a Zeiss photomicroscope for proper orientation prior to the electron microscopic examination. Ultrathin sections obtained with a Porter-Blum and LKB ultramicrotomes, were stained with uranyl acetate and lead citrate and examined in a JEOL 100B electron microscope. Observations were made using intermediate magnifications ranging from 30-60.000 X. For ethical reasons proper human control specimens were not studied. Normal animal tissue (mice, rats and teleost fishes) similarly processed have been used to control sources of artifacts of the human sampling procedure for transmission electron microscopy.

RESULTS

In all cases examined the human brain parenchyma exhibited vasogenic brain edema due to the physical fragmentation of the blood brain barrier induced by the intensity of the traumatic head injury. The vasogenic brain edema was primarily characterized by enlargement of the extracellular space, and secondarily by ischemia-induced cytotoxic intracellular edema. Swollen clear mitochondria were generally observed in the soma of non-pyramidal neurons in all cases under study, and characterized by the low electron density of the mitochondrial matrix (Fig 1).

Note the dilated rough endoplasmic reticulum (ER). The nucleolar organized fibrillar center (FC) and the dense fibrillar center (DFC) .The granular component (GC) also is distinguished. In the degenerated myelinated axons the mitochondria exhibited in addition discontinuities of outer mitochondrial membrane (Fig.2).

We found varicose swollen dendrites exhibiting clear, round and elongated mitochondria with fragmented cristae (Fig.3).

The axodendritic contacts localized in the vicinity of non-pyramidal neurons showed moderate edema of presynaptic ending mitochondria, and marked edematous changes of postsynaptic ending mitochondria (Fig. 4).

The axosomatic synaptic contacts on non-pyramidal neurons also showed edematous clear presynaptic mitochondria (Fig.5).

Table I. Neurosurgical Study

CASE NO.	AGE AND SEX	CLINICAL DATA	DIAGNOSIS	EDEMA	CORTICAL BIOPSY AND SITE OF INJURY	EVOLUTION TIME OF BRAIN INJURY
1. JP (CCG29)	14 y, M	Contusion and cave-in-fracture of frontal region, transitory loss of consciousness	Contusion and cave-in fracture of frontal region.	severe	Left frontal cortex. Focal Region.	1 day
2. HRF (CCH17)	18 y, F	Severe frontal contusion cave-in fracture in road accident, loss of consciousness. Convulsive crisis.	Severe frontal contusion	Severe	Left frontal cortex. Focal Region.	8 days
3. JRCR (CCH31)	69 y, M	Falling from his own height, chronic alcoholic patient presented headache, diminution of muscle strength of lower extremities and right arm, temporary loss of consciousness, dysarthria, anisocoria.	Brain trauma. Left frontoparietal occipital subdural hematoma	Severe	Left parietal cortex. Focal and Perifocal Regions.	16 days
4. JM (CCH21)	58 y, M	Road accident. Patient showing contusion and hematoma of left temporo-parietal region. Clouded sensorium, temporospatial disorientation. Left mydriasis.	Brain trauma Left parietooccipi-tal subudral hygroma	Severe	Left parieto temporal cortex. Focal Region.	19 days
5. OP (CCH30)	60 y, F	Head injury in traffic accident, fracture of both legs, state of coma, abolition of reflexes. Left mydriasis. After recovery showed disorders of behavior. (Post-traumatic confusional syndrome)	Brain trauma. Subdural hygroma	Severe	Right parietal cortex. Focal Region.	25 days
6. ANG (CCH18)	39 y, M	Loss of consciousness after falling from a running truck, headache. Left hemiparesis, papilledema.	Brain trauma. Right parieto temporal subdural hematoma	Severe	Right temporo parietal cortex. Focal Region.	8 months
7. LCS (CCH64)	20 y, F	Frontal headache	Brain trauma. Left frontal Subdural hematoma	Severe	Left parietal cortex. Focal Region.	6 days
8. IJA CCH61	27 y, F	Patient hit with a stick on a fighting street. Brain trauma. Biparietal fracture. Reintervened by biparietal craneoplastic surgery.	Biparietal trauma.	Severe	Right parietal cortex. Focal Region.	8 months
9. ASCR (CCH22)	26 y, M	Falling from his own height. Skull trauma in right temporo-parietal region, tonic clonic convulsion, and disorders of behavior	Brain trauma. Right parieto temporal. Subdural hematoma. Brain contusion.	Severe	Right temporo parietal cortex. Focal Region.	7 months
10. PDM. (CCH27)	21 y, M	Falling from a light post, coma, bilateral papilledema.	Brain trauma Right epidural hematoma.	Severe	Right temporal Cortex. Focal Region.	1 day

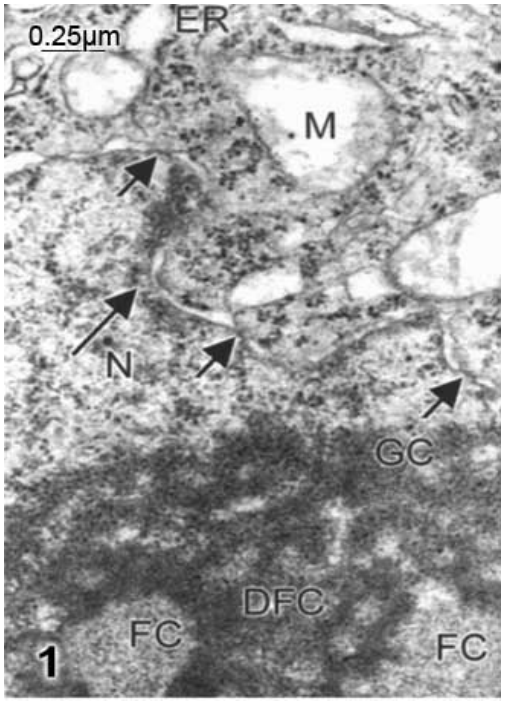

Fig. 1. Brain trauma. Clear swollen mitochondria (M) at the perinuclear region of a non-pyramidal nerve cell (NP), also seen: endoplasmic reticulum (ER), nucleolar organized fibrillar center (FC), is the dense fibrillar center (DFC) and the granular component (GC). The long arrow labels the perinuclear cistern, and the short arrows the nuclear pores.

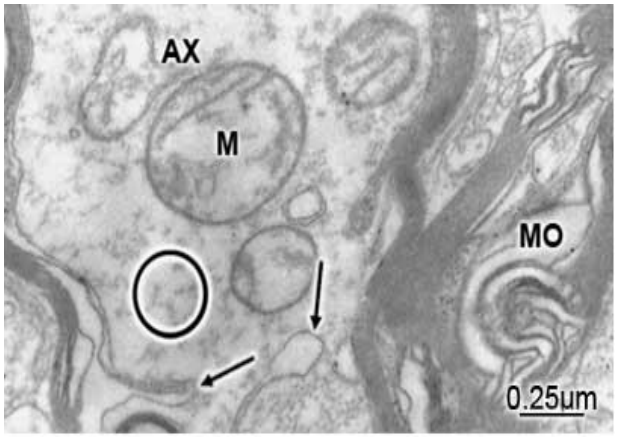

Fig. 2. Brain trauma. Degenerated myelinated axon (AX) showing severely edematous mitocondria (M). Deep invaginations of axolemmal membrane (arrows) and outer myelin ovoids (MO) also are distinguished. The circle label the fragmented cytoskeletal structures.

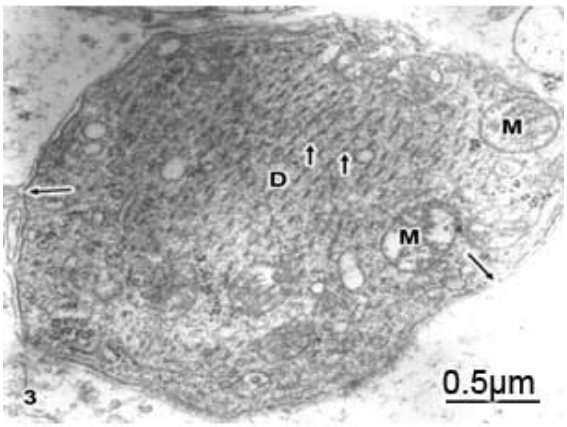

Fig. 3. Brain trauma. Cross section of a swollen dendritic process (D) exhibiting edematous clear mitochondria (M). .The tangential sections of microtubules (short arrows), and the disrupted limiting plasma membrane (long arrows) also are seen.

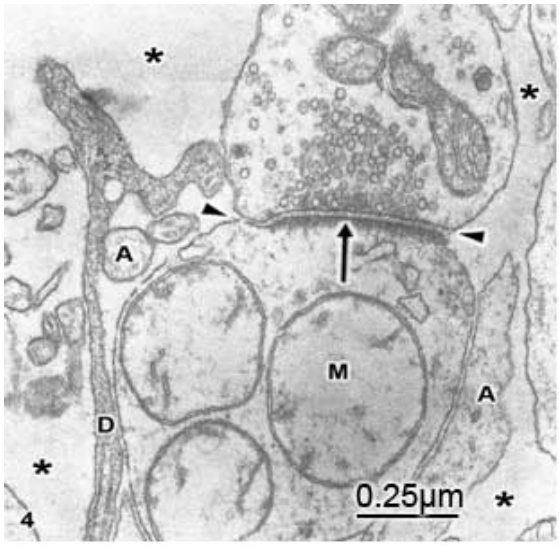

Fig. 4. Brain trauma. Axodendritic contact in the edematous neuropil showing notably edematous mitochondria (M) with few fragmented cristae in the postsynaptic ending and moderately swollen mitochondria in the presynaptic ending. Fine dendritic profiles (D) and astrocytic processes (A) also are distinguished. The arrowhead indicates the synaptic cleft. Note the notably enlarged extracellular space (asterisks).

In the severely edematous neuropil the swollen dense and hypertrophic astrocytes displayed swollen clear mitochondria, dense swollen mitochondria, and degenerated mitochondria (Fig. 6), suggesting a major vulnerability of astrocytic mitochondria to anoxic-ischemic conditions.

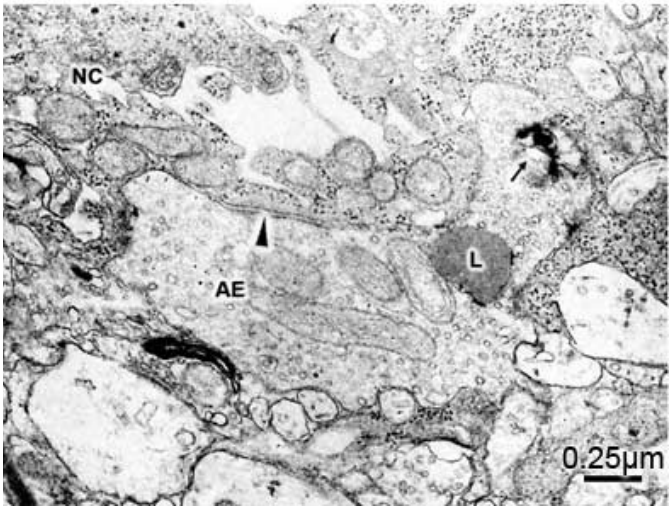

Fig. 5. Brain trauma. Axosomatic ending (AE) on a non-pyramidal nerve cell (NC) showing four edematous mitochondria in the presynaptic axosomatic ending (AE) containing electron dense necrotic material. (short arrow). A lysosome (L) also is seen.

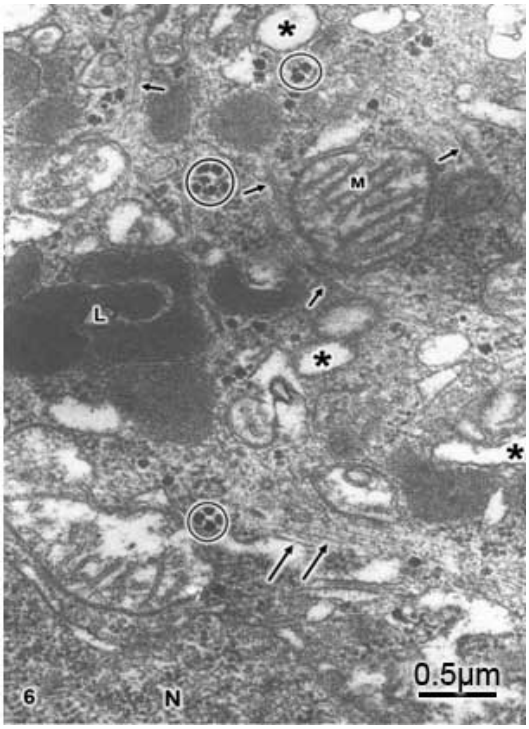

Fig. 6. Brain trauma. Dense hypertrophic astrocyte exhibiting swollen clear mitochondria with fragmented cristae (M). The circles label the glycogen granules. Lobulated lysosomes (L) also are seen. The asterisk point out the enlarged endoplasmic reticulum profiles. The long arrows label actin-like filaments. The short arrow indicates the microtubules.

The perivascular astrocytic end-foot contains also the same mitochondrial structural pattern observed in the cell body (see Fig. 7).

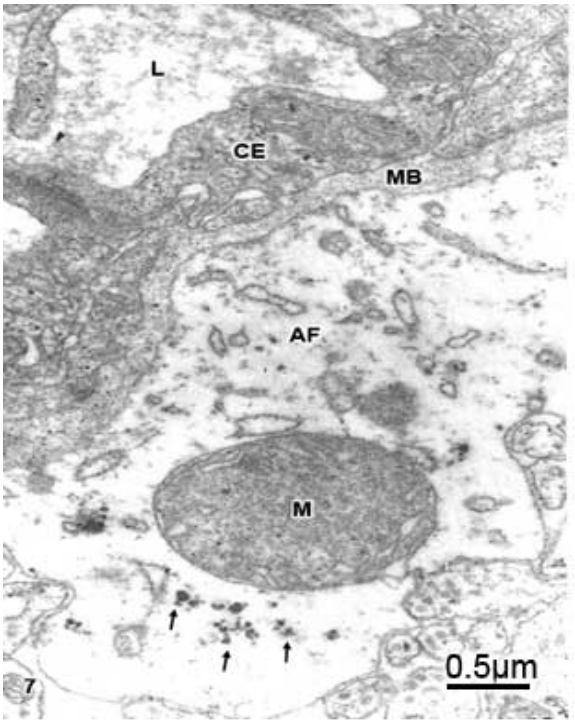

Fig.7. Brain trauma. Cortical capillary wall showing the attached perivascular astrocytic end-foot (AF) containing a dense degenerated mitochondria with fragmented cristae. The arrows indicate the glycogen granules. The capillary lumen (L), endothelial cell (EC) and the basement membrane (BM) also are noted.

In very severe vasogenic edema with notably enlargement of extracellular space, isolated and fragmented mitochondria, surrounded by dense proteinaceous edema fluid, were observed showing similar structural patterns of swollen mitochondria. These isolated mitochondrial are released from necrotic nerve cell death (Fig. 8).

In oligodendroglial cells swollen mitochondria with a clear matrix and edematous cristae were noted (Fig. 9).

At the level of damaged open or collapsed cortical capillaries, the endothelial cells and the pericyte cells showed notably swollen mitochondria. The open or collapsed capillaries exhibited mitochondria with intact or fragmented cristae, and continuous or disrupted inner

and outer mitochondrial membrane, mainly localized at the organelle zone of endothelial cells. Dense degenerated mitochondria were found in collapsed capillaries with signs of increased transendothelial transport (Figs. 10 and 11).

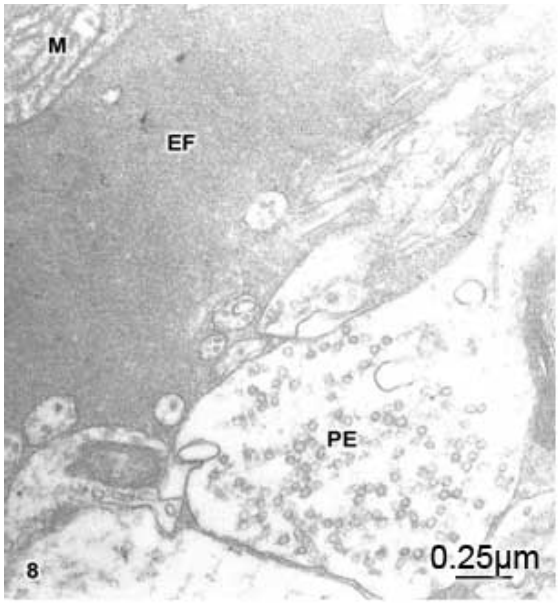

Fig. 8. Brain trauma. Isolated and swollen clear mitochondrion (M) floating in the electron dense proteinaceous edema fluid (EF) localized in the enlarged extracellular space. A swollen and degenerated presynaptic ending (PE) also is seen.

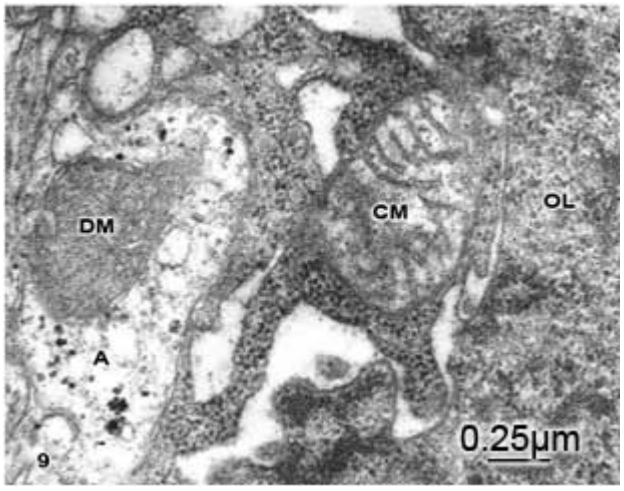

Fig. 9. Edematous oligodendroglial cell (OL) illustrating a swollen clear mitochondria (CM). A dense and swollen mitochondria (DM) is observed in the neighboring astrocytic (A) cytoplasm.

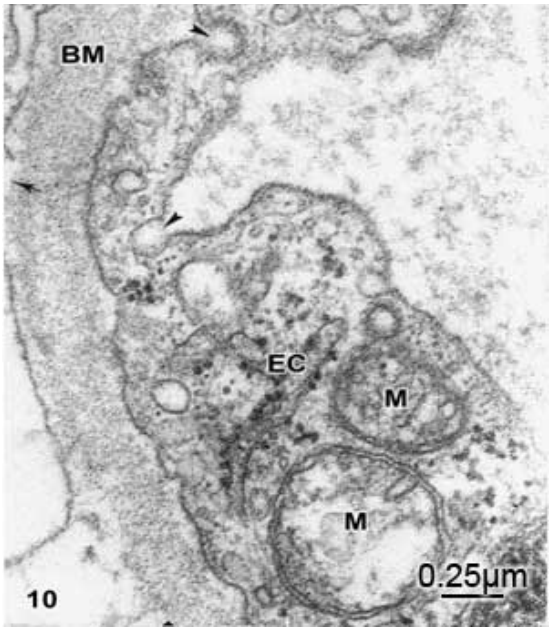

Fig. 10. Brain trauma. Swollen mitochondria (M) depicting matrix vacuolization and cristolisis (M) in the organelle zone of endothelial cell (EC). Note the swollen basement membrane (BM). The arrowheads indicate micropinocytotic and coated vesicles. The short arrow shows the disrupted astroctic end-foot membrane.

Mitochondrial damage was observed promoting or inducing several types of nerve cell death. Dense ischemic neurons were frequently observed (Fig. 12). Apoptotic astrocytes were distinguished containing swollen mitochondria with partial disappearance and fragmentation of cristae (Fig. 13). Ischemic and hydropic oligodendrocytes also were seen (Fig. 14).

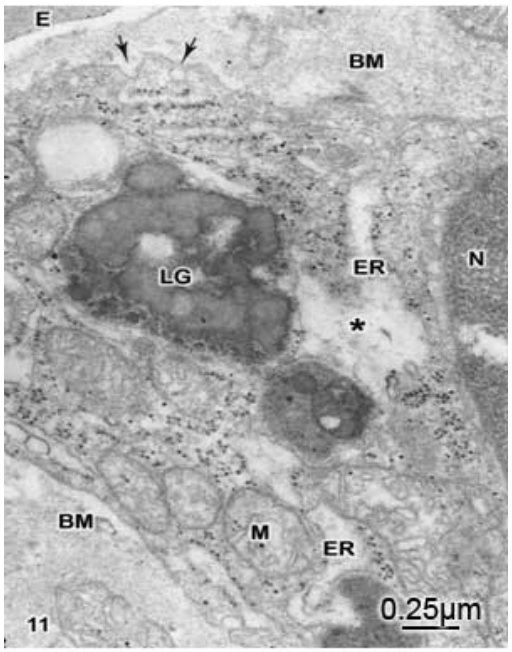

Fig. 11. Swollen pericyte embedded within a remarkably edematous capillary basement membrane (BM) showing clear swollen mitochondria (M). Note the dilated endoplasmic reticulum (ER), the thickened basement membrane (BM), lipofucsin granules (LG), and pericyte nucleus (N). The asterisk labels lacunar enlargement of endoplasmic reticulum. The arrows points out open micropinocytotic vesicles discharging into the basement membrane. The arrowheads show the dissociated perycte cytoplasm from the basement membrane.

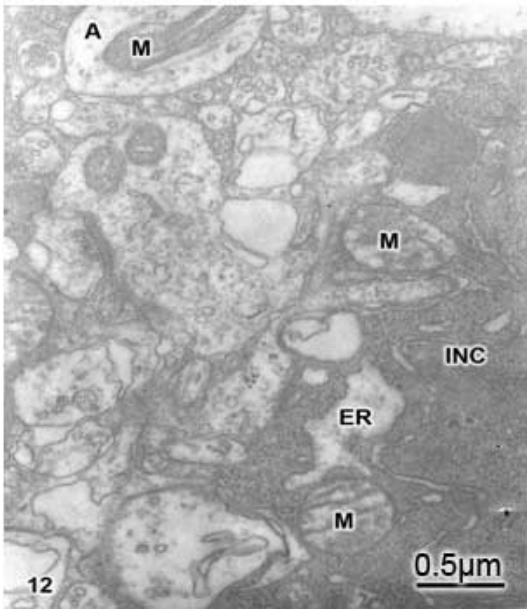

Fig. 12. Dark ischemic nerve cell death (INC) showing edematous mitochondria (M). Note the lobulated nucleus and the enlarged endoplasmic reticulum (ER). A dense mitochondria (M) is observed in an astrocytic cytoplasm (A) at the level of the neighboring neuropil.

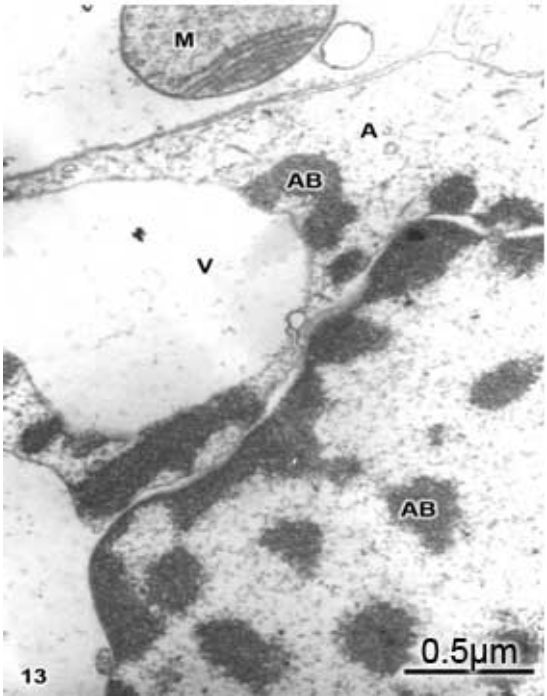

Fig. 13. Brain trauma. Apoptotic astrocyte containing apoptotic bodies (AB) in the nucleoplasm and the cytoplasm. An edematous mitochondrion (M) with partial disappearance of cristae is observed in its neighboring astrocytic process. A huge cytoplasmic vacuole (V) is seen surrounded by the apoptotic bodies.

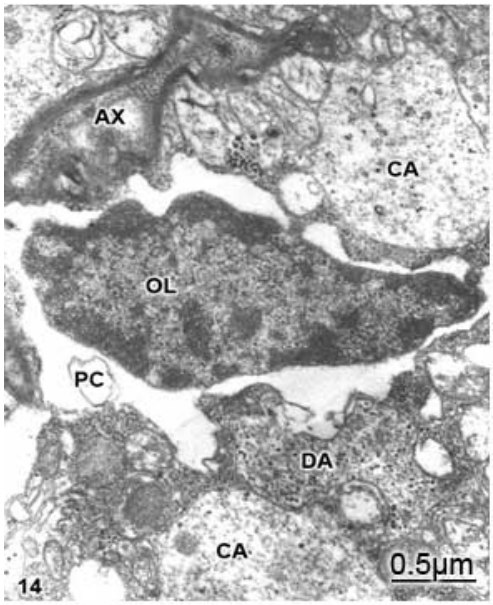

Fig. 14. Ischemic and hydropic oligodendrocyte (OL) showing lacunar enlargement of perinuclear cistern (PC) and a dense band of rejected electron dense cytoplasm associated to a degenerated myelinated axon (AX). Dense (DA) and clear (CA) astrocytic cytoplasm also are observed.

DISCUSSION

Light and dense mitochondria have been earlier reported by Ikrenyi et al. [6] in human postmortem specimens. These authors correlated the light type of mitochondria with the non-functional homogeneous type, and the dense form of mitochondria with the functional state. However, postmortem specimens introduce artifactual damage at the electron microscopic level due to the anoxic changes after patient death. Therefore, in the present study cortical biopsies wee used, taken in the neurosurgical room at the beginning of neurosurgical treatment, and immediately gluraldehyde-fixed in the surgical room, in order to avoid delayed fixation and postmortem changes. Solenski et al. [21] reported dense cortical neuronal mitochondria exposed to severe ischemic/reperfusion conditions, and increasing loss of mitochondrial density with pronounced swelling in permanent ischemia. The swollen clear mitochondrial type herein described are related with both, the complicated brain trauma and the secondary ischemic process. In addition, in severe brain edema we have found fragmentation of cristae, which are extension of inner mitochondrial membrane. The mitochondria show significantly different pattern of injury expressed by notably electron density changes of mitochondrial matrix. This observation is probably related with mitochondrial matrix protein aggregation, which could be responsible by its osmiophilic property at the electron microscopy level. The fragmentation of the mitochondrial cristae suggests that oxidative phosphorylation of ADP, the precursors of the high-energy phosphate bond of ATP, no longer occurs. In addition, it means that an interruption of mitochondrial membrane intracellular transport occurs, which cause respiration-dependent extrusion of H^+ and accumulation of Ca^{2+} from the cytoplasm [30]. During ischemia the lack of oxygen blocks oxidative metabolism so there is no enough energy to maintain the membrane potential require to drive Ca^{2+} uptake into the mitochondria]. However, in brain edema, mitochondria can accumulate excessive amounts of Ca^{2+} and become overloaded [31]. When this occurs, mitochondria undergo a high permeability transition of the inner mitochondrial membrane [32,33], release Ca^{2+}, and become swollen and uncoupled [34]; thereby loosing the ability to produce ATP, and leading to nerve cell death. Therefore, neuronal death. by necrosis or apoptosis depend of mitochondrial function [35], a fact clearly demonstrated in the present study. Presumably the catastrophic brain injury induced a high conductance permeability transition pore or megachannel [36,37] in the inner mitochondrial membrane, which causes mitochondrial swelling. In this context, mitochondrial swelling should be considered as an epiphenomenon [38] preceding nerve cell death.

Glutamate excitotoxicity is the process whereby a massive glutamate release occurs in the central nervous system in response to ischemia or related trauma leading to a delayed, predominantly ischemic cell death of neurons, as illustrated in the present study. Mitochondria accumulate much of the post-ischemic calcium entering the neurons via the cronically activated N-methyl-D-aspartate receptors contributing to excitotoxicity [31,39,40].

Nitric oxide and its derivative peroxinitrite inhibit mitochondrial respiration (complexes I, II and V). NO-induced inhibition of respiration in brain nerve terminals results in rapid glutamate release, which might also contribute to neurotoxicity [41]. Peroxinitrite also causes opening of the mitochondrial permeability transition pore, resulting in release of cytochrome C, which might then trigger apoptosis [42]

Impaired brain mitochondrial respiration and decreased cytochrome oxidase activity have been found after delayed onset of neurologic deterioration following anoxia/ischemia [11,12]. Novikov and Sharov [43] and Sharov and Novikov [15] reported that the rate of oxidation decrease in mitochondria in toxic and traumatic edema.

Lipid peroxidation occurs also in brain edema following ischemia and hypoxia [44–47], which could be responsible for the mitochondrial membrane damage. The mitochondrial generation of superoxide anions is enhanced during anoxia and reoxigenation [41,47]. Mitochondrial electron transport also generates reactive oxygen intermediates (ROI). A large increase of ROI induce collapse of mitochondrial membrane potential and neuronal cell death [48–52]. The elevated intracellular Ca^{2+} and exposure to fatty acids, which alter the physical properties of mitochondrial membranes and inhibition of mitochondrial respiratory components, may enhance this leak of ROI from mitochondria. Cytochrome C is released from mitochondria into the cytosol contributing to mitochondrial dysfunction and promoting ischemic neuronal injury and delayed nerve cell death. Releasing of cytochrome C induces to downstream consequences of specific caspase activation and apoptosis. The amplification of oxidative stress and Ca2+ loading culminates in necrotic cell death [52–58].

Mitochondrial are therefore pivotal regulators of cell death through their role in energy production and calcium homeostasis, by their capacity to release apoptogenic proteins, and to produce reactive oxygen species [59].

CONCLUSIONS

Three morphological structural patterns of injured mitochondria are found in the traumatic brain injuries at the level of edematous cerebral cortex: clear swollen mitochondria, swollen dense mitochondria dense, and degenerated mitochondria. The mitochondrial damage induced apoptotic and ischemic nerve cell death. And it has been postulated as marker of lethal nerve cell injury. The vasogenic brain edema, the associated secondary ischemic damage of brain parenchyma, glutamate cytotoxicity, calcium overload, and peroxidative damage have been correlated with nerve cell mitochondriopathy

ACKNOWLEDGEMENT

This paper has been carried out by a subvention obtained for CONDES/LUZ.

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