CONTRIBUTIONS OF GOLGI APPARATUS AND MYELINATED AXON DERANGEMENT TO THE SYNAPTIC DEGENERATION IN TRAUMATIC HUMAN BRAIN EDEMA. AN HYPOTHESIS.

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ABSTRACT
The goal of this paper is to correlate the traumatic injury-induced edematous changes and the derangement of nerve cell Golgi apparatus and interrupted axonal flow with the synaptic degeneration. We postulate the following hypothesis: The damaged of the nerve cell Golgi apparatus, normally involved in the storage of most synaptic proteins and source of synaptic vesicles, and the interrupted axonal flow are related with synaptic degeneration in the traumatic brain edema? Cortical biopsies of cerebral cortex of 10 patients with severe traumatic head injuries complicated with subdural and extradural hematoma or hygroma were examined. They were immediately fixed in buffered glutaraldehyde fixative in the surgical room and conventionally processed for transmission electron microscopy. A notably edematous Golgi apparatus featured by marked enlargement of Golgi stack cisternae in pyramidal nerve cells, and with minor edematous changes in the perineuronal oligodendroglial cells were observed. Extremely swollen and fragmented Golgi complexes were found in non-pyramidal neurons and endothelial cells. Beaded shaped degenerated myelinated axons did not exhibit axonal flow of mitochondria and Golgi apparatus derived vesicles. Coexisting degenerated axodendritic contacts were observed in the neighboring edematous neuropil. Conclusions: The electron microscopic images suggest that the derangement of Golgi complex and the interruption of axoplasmic flow in the traumatic brain injury primarily participate in the synaptic degeneration, and secondarily the ischemic process, calcium overload, glutamate and hemoglobin citotoxicity, and lipid peroxidation.

Keywords: Golgi apparatus, myelinated axons, synaptic degeneration, electron microscopy

RESUMEN
El objetivo del presente trabajo es correlacionar la probable relación entre el daño del complejo de Golgi y la interrupción del flujo axoplásmico en el edema cerebral traumático con la degeneración sináptica. Postulamos la siguiente hipótesis: El aparato de Golgi, normalmente envuelto en el almacenamiento de proteínas sinápticas y en el origen de las vesículas sinápticas, y la interrupción del flujo axoplásmico pueden en el daño sufrido por edema traumático inducir primariamente la degeneración sináptica?. Se examinaron al microscopio electrónico 10 biopsias corticales de pacientes con traumas cerebrales severos complicados con hematomas subdurales, extradurales e higromas. Las biopsisas corticales se fijaron inmediatamente en solución tampón de glutaraldehido en la sala operatoria y procesadas en el laboratorio según la técnica convencional para microscopía electrónica de trasmisión. Se observaron aparatos de Golgi con edema marcado y fragmentación de los grupos de cisternas aplanadas en neuronas piramidales y cambios edematosos menores en la oligodendroglía perineuronal. Aparatos de Golgi edematosos, y fragmentados se observaron en las neuronas no piramidales y células endoteliales. Los axones mielínicos varicosos y degenerados no mostraron evidencias de flujo axonal de mitocondrias y de vesículas originadas del aparato de Golgi. Se observaron contactos sinápticos axodendríticos degenerados en la neuropila vecina. Conclusión: las imágenes electrono-microscópicas sugieren que la alteración del complejo de Golgi, la degeneración de los axones mielínicos y la interrupción del flujo axoplásmico en el edema cerebral traumático participan primariamente en la degeneración sináptica, y secundariamente son afectados por el proceso isquémico, la elevación del calcio, citotoxicidad por glutamato y hemoglobina, y la peroxidación lipídica.

Palabras claves: Aparato de Golgi, axones mielínicos, degeneración sináptica, microscopía electrónica.

INTRODUCTION

Most pathological alterations of the Golgi apparatus have been reported in a large variety of pathological conditions, such as human brain tumors [1], type-C Niemann-Pick disease [2], aging and in Alzheimer's disease [3-6], a variety of non-nervous diseases involving altered secretory activity (albuminemia, lipoproteinemias), cell surface pathology (cancer) and viral diseases [7,8] human congenital hydrocephalus [9], amyotrophic lateral sclerosis [10-15], leptomeningeal lymphoma, leukemia, multiple myeloma, multiple sclerosis , tumoral prolactin cells in culture cells [16], corticobasal degeneration and Creutzfeldt-Jacob disease [17], human olivary hypertrophy [18], X-linked spinal and bulbar muscular atrophy [19], tau-induced degeneration in astrocytes [20], a subset of myopathies [21], following intracerebroventricular injection of streptozotocin in rats [22], late onset cerebellar and sensory ataxia in mice with inactivated abcd1 gene [23], neurodegenerative diseases, brain ischemia, apoptotic and necrotic cell death after ischemia [24-26]. In earlier paper we have analyzed the edematous changes of Golgi apparatus in brain trauma, tumos and congenital malformations [9].

In the present paper we describe by means of conventional transmission electron microscopy, using human cortical biopsies, the edematous changes and fragmentation of Golgi apparatus, and the interrupted axoplasmic flow in severe and complicated human traumatic brain injuries, and we postulate their relationship with the synaptic degeneration.

MATERIALS AND METHODS

Samples of cerebral cortex of 10 patients with traumatic head injury complicated with subdural and extradural hematoma or hygroma were used in the present study. Cortical biopsies were performed according to the basic principles of the Helsinki Declaration, and the permission obtained from Biological Research Institute Ethical Committee. Clinical data, diagnosis, biopsy region and degree of brain edema appear listed in Table No. 1. 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 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 ultramicrotoms were stained with uranyl acetate and lead citrate, and examined in a JEOL 100B electron microscope. Observations were made using intermediate magnifications ranging from 24.000-60.000 X. For ethical reasons proper human control specimens were not studied. Normal cerebral cortex animal tissue (mice, rats) were simultaneously processed as "controls", to discard sources of artifacts of the human sampling procedure for transmission electron microscopy.

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	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	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	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 fronto-parieto-occipital subdural hematoma.	Severe	Left parietal cortex. Focal and perifocal region.	16 days
4.JM	58 y, M	Road accident. Patient showing contusion and hematoma of left temporo-parietal region. Clouded sensorium, temporospatial disorientation. Left mydriasis.	Brain trauma Left parieto-occipital subdural hygroma.	Severe	Left parieto-temporal cortex. Focal Region.	19 days
5. OP	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	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	20 y, F	Brain trauma Frontal headache	Brain trauma. Left frontal subdural hematoma	Severe	Left frontal cortex. Focal Region.	6 days

8. IJA	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	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.	Severe	Right temporal cortex. Focal Region.	7 months
10. PDM.	21 y, M	Falling from a light post, coma, bilateral papilledema.	Brain trauma Right epidural hematoma.	Severe	Right temporal Cortex. Focal Region.	1 day

RESULTS AND DISCUSSION

In a patient with brain trauma complicated with a left fronto-parieto-occipital subdural hematoma (Case No.3), a notably edematous Golgi complex was observed featured by marked enlargement of Golgi stacked cisternae in the perinuclear region of non-pyramidal nerve cells. The rough endoplasmic reticulum canaliculi also showed lacunar dilation (figure1).

In another patient with a head traumatic injury associated with a left parieto-occipital subudral hygroma (Case No, 4), a swollen Golgi apparatus was found. The rough endoplasmic reticulum exhibited moderate enlargement, and partially degranulated limiting membranes (figure 2).

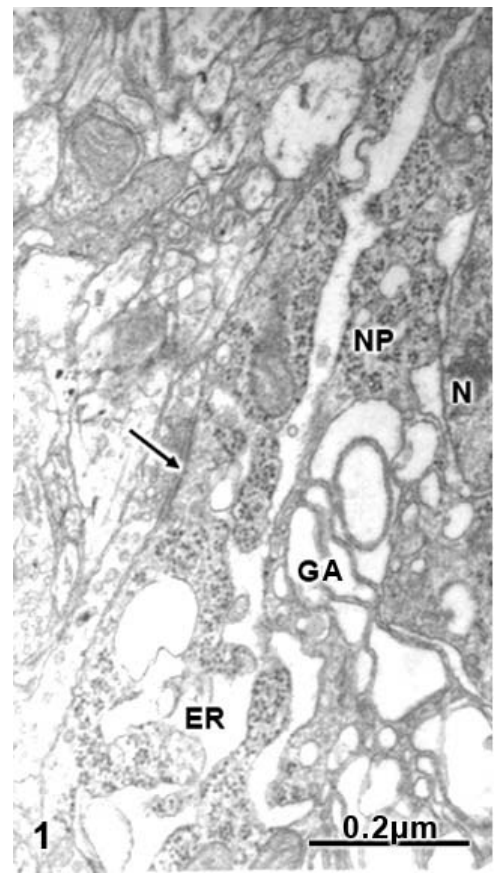

Fig.1. Case No.3. Brain trauma. Left fronto-parieto-occipital subdural hematoma. Left parietal cortex. Non-pyramidal neuron (NP) showing lacunar enlargement of Golgi apparatus (GA), and endoplasmic reticulum (ER). The nucleus (N), and an axosomatic synapse (arrow) also are distinguished.

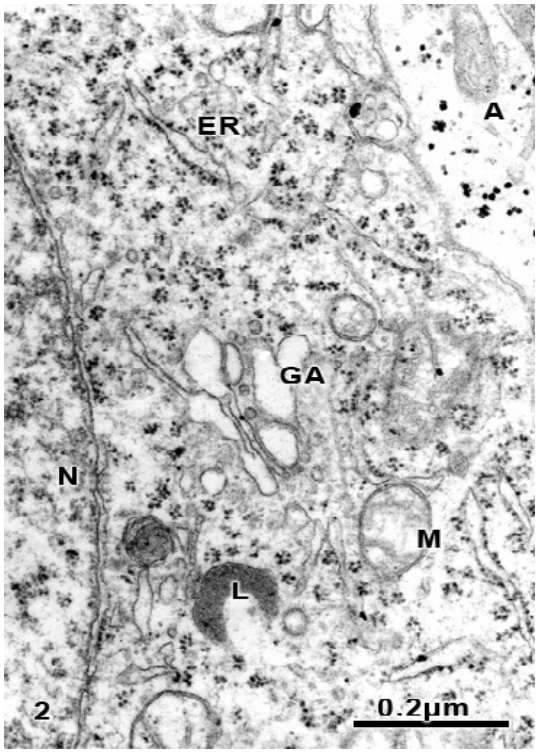

Fig. 2. Case No.4. Brain trauma. Left parieto-occipital subudural hygroma. Left parietal cortex. Swollen non-pyramidal neuron showing a severely edematous Golgi apparatus (GA), the nucleus (N), a partially degranulated rough endoplasmic reticulum (ER), swollen mitochondria (M), and a lobulated lysosome (L). An astrocytic cytoplasm (A) also is seen.

In a patient with head contusion trauma of frontal region (Cases No. 1 and 2), we found swollen non-pyramidal neuron displaying edematous and fragmented Golgi apparatus with marked enlargement of Golgi endoplasmic sacs (figure 3).

In a patient with a very severe brain traumatic lesion complicated with a right parieto-temporal subdural hematoma (Case No. 6), we observed beaded shaped and degenerated myelinated axons, in which the axonal flow of mitochondria and Golgi apparatus derived vesicles appear interrupted (figure 4).

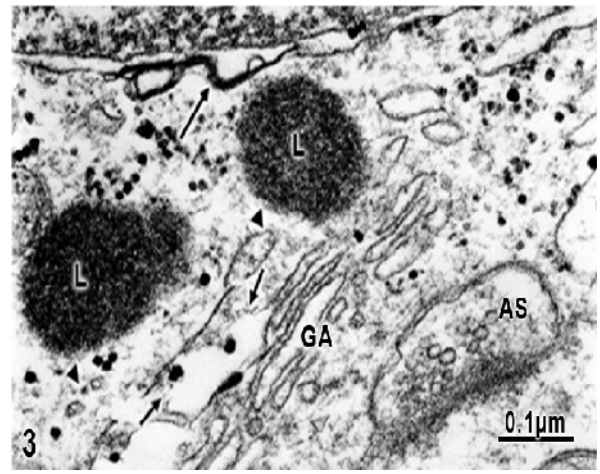

Fig 3. Case No.7. Brain trauma. Left frontal subdural hematoma. Left frontal cortex. Non-pyramidal neuron showing an edematous and fragmented Golgi apparatus (short arrows). Note the osmiophilic and damaged nuclear envelope (long arrow). The lysosomes (L) show a discontinuous globular limiting membrane (arrowheads). An asymmetric synaptic contact (AS) also is observed.

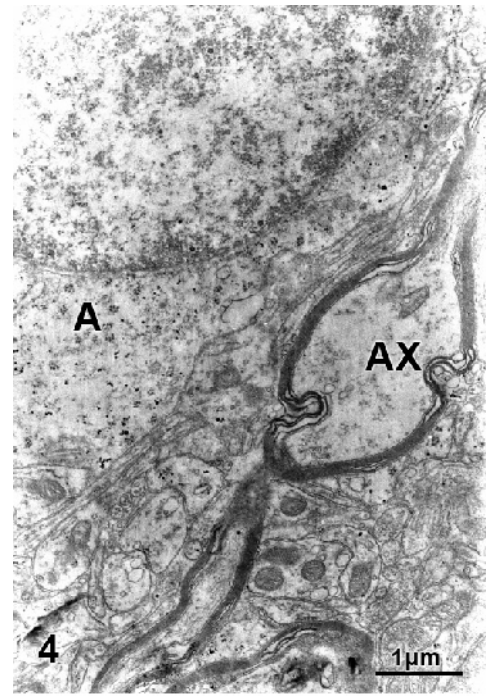

Fig. 4. Case No.6. Brain trauma. Right parieto-temporal subdural hematoma. Right parietal cortex. Varicose and degenerated myelinated axon (AX) in the vicinity of a swollen astrocyte cell (A) depicting the granular disintegration of microtubules and neurofilaments, and the absence of mitochondria and Golgi derived vesicles, demonstrating the obstructed axonal traffic of Golgi vesicles from the Golgi compartment to the synaptic ending.

In a marked head trauma located at the frontal region and associated with a left frontal subdural hematoma (Case No. 7), severely degenerated myelinated axons were observed showing an abnormal deposit of degenerated mitochondria and granular disintegration of cytosketal structures in the axoplasm, revealing interrupted axonal traffic of Golgi vesicles and mitochondria moving to the synaptic endings. Due to the damage and disassembly of microtubules, that act as guiding structures of fast axonal axoplasmic flow, the axonal traffic of Golgi derived vesicles is interrupted (figure 5).

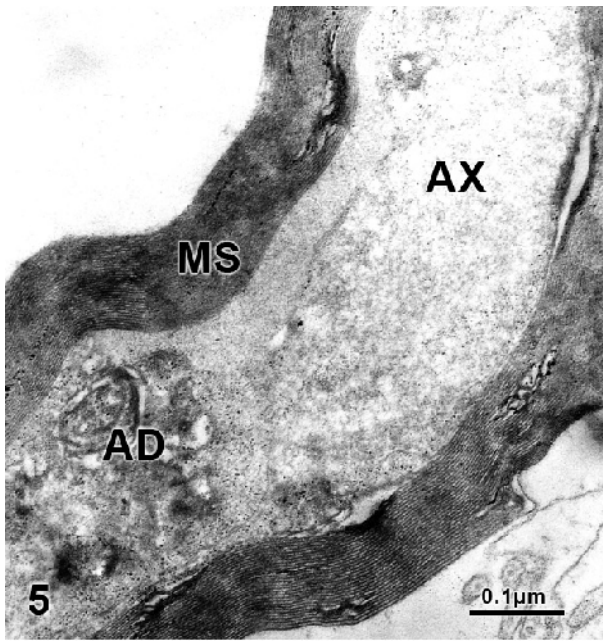

Fig. 5. Case. No. 7. Brain trauma. Left frontal subdural hematoma. Left frontal cortex. Severely degenerated myelinated axon (AX) showing in the axoplasm the abnormal deposit (AD) of disintegrated cytosketal structures and mitochondria, that evince the non-circulating axonal flow of mitochondria and Golgi vesicles. The damage myelin sheath (MS) featured by enlargement of intraperiod space also is seen.

In a patient with a severe traumatic injury of right parietal and temporal regions and complicated with a parieto-temporal subdural hematoma (Case No. 9), very severe degenerated myelinated axons were found coexisting with degenerated axodendritic synaptic contacts, which displayed the clear and dark-type of degeneration of pre- and post synaptic endings (figures 6 and 7).

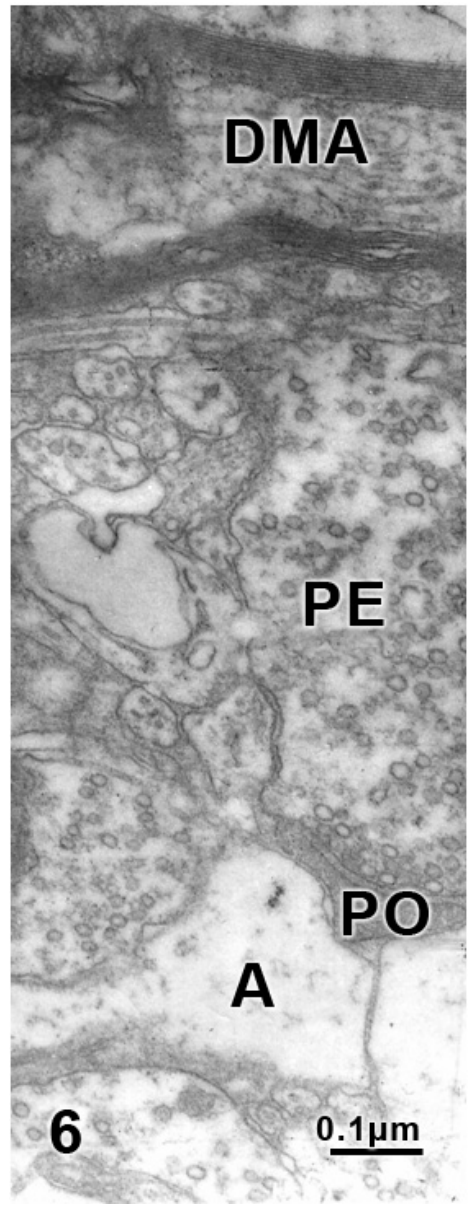

Fig. 6. Case No.7. Brain trauma. Right parieto-temporal. subdural hematoma. Right temporal cortex. Degenerated axodendritic synaptic contact (arrow) showing a swollen and clear presynaptic ending (PE) synapsing with a dark postsynaptic terminal (PO) in the vicinity of a notably degenerated myelinated axon (AX). A swollen astrocytic cytoplasm (A) also is observed.

In a patient with a brain trauma of right parietal cortex complicated with a subdural hygroma, and very severe edematous cortical regions (Case No. 5), degenerated synaptic endings were observed characterized by clumping, and disappearance of synaptic vesicles (figure 8).

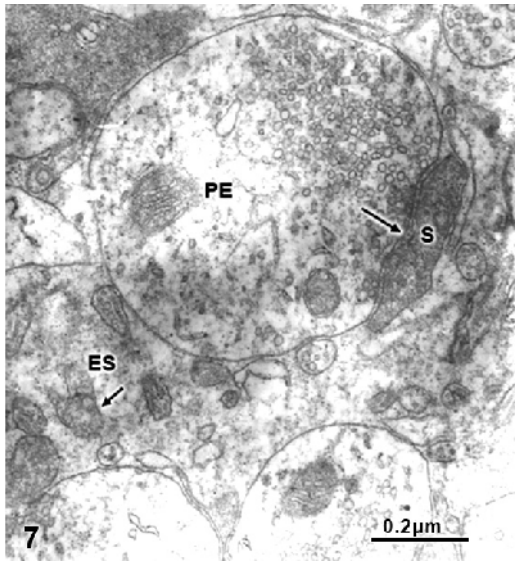

Fig. 7. Case No.9. Brain trauma. Right temporal cortex. Right parieto-temporal. subdural hematoma. Right temporal cortex. Light and severely swollen presynaptic endings (PE) making axospinodendritic contact (long arrow) with a dark dendritic spine (S). Note the presence of proteinaceous edema fluid in the distended extracellular space (ES) containing cell debris (short arrow).

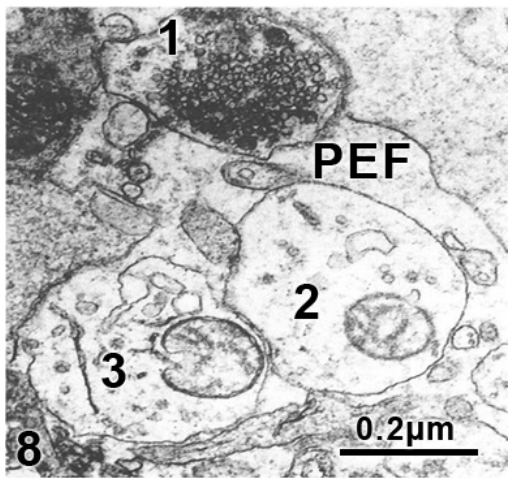

Fig. 8. Case No.10. Severe brain trauma complicated with a subdural hygroma. Right parietal cortex. Clear degenerated and isolated pre-synaptic endings (1, 2, 3) floating in a lake of amorphous proteinaceous edema fluid (PEF), and exhibiting different degrees of synaptic degeneration. Synaptic ending labeled 1 shows clumping of synaptic vesicles, and those labeled 2 and 3 exhibit disappearance of most synaptic vesicles.

In the present paper we postulate the like hypothesis of a relationship between Golgi apparatus pathology, interruption of axoplasmic flow and synaptic degeneration. Most biochemical, cell and molecular biology studies carried out in the last two decades in model experimental animals dealing with the role of Golgi apparatus in synaptic formation during synaptogenesis and mature conditions demonstrated the intimate relationship between synaptic proteins formation in the Golgi apparatus, axonal transport of Golgi derived vesicles, and their migration to the synaptic endings. The relationship between the Golgi apparatus and synaptic contacts in normal conditions in experimental animal have been previuosly reported by numerous studies. Electron microscopic immunocytochemical basic investigations have shown Rab6p in post-Golgi transport, and synaptophysin release from the Golgi apparatus in a vesicular form, after glycosilation, and then transportation to nerve endings by a mechanism that require the integrity of microtubules [27-29]. It has been postulated that the synaptic vesicles originate directly from the trans-Golgi network, and early endosomes [30,31). Newly synthesized Golgi vesicles are transported by fast axonal flow to their target, such as nerve cell limiting plasma, axolemmal membranes, and synaptic vesicles [32,33]. According to Dresbach et al. [34], the transport via Golgi-derived vesicles is essential for delivery of cytomatrix proteins to the synapse, and the Golgi transit is an obligatory step for subcellular trafficking of distinct cytoplasmic scaffolding proteins. Morgan et al. [35] demonstrated that SNAP-25 is a neuronal SNARE protein present in the trafficking Golgi vesicles and synaptic vesicles. N-ethilmaleimide-sensitive factor attachment protein receptor (SNARE), including syntaxin 5, GOS-28, membrin, rsec22b, and rbet1 are localized into the Golgi stacks [36]. Taking into account these cytochemical studies, we have postulated the present hypothesis. In the patients under study the severe traumatic brain edema induces swollen, and fragmentation of Golgi apparatus, and therefore an interference with the constitutive and regulated secretions

of Golgi synaptic proteins in the swollen nerve cells. The anoxic-ischemic conditions of nerve tissue in complicated traumatic brain injuries would alter the pos-translational modifications of proteins within the Golgi apparatus. Consequently, due to the damage of Golgi apparatus, another phenomenon, such as the insertion of integral membrane proteins, including ion channels and receptor molecules, into the plasma membrane is disturbed causing edematous changes and fragmentation of the plasma membrane [37]. According to Gonatas et al. [38], the process of regulated secretion, which is acutely stimulated by external factors, such as trauma and tumors, also is altered. In addition, the increased levels of calcium in ischemic conditions open the voltage-dependent calcium channels and increase the regulated secretion process [39]. The damage of the axolemmal membrane, the disassembly of microtubules, and the cluster of axonal organelle in the degenerated myelinated axons [40] interrupt the axonal traffic of Golgi synaptic vesicles inducing the clear and dark type of synaptic degeneration. These findings strongly suggest that swollen mitochondria [41] and Golgi apparatus edema and fragmentation could be considered as an early markers of nerve cell injury and degeneration.

In the damaged Golgi apparatus herein described presumably we are dealing with two successive pathological events, involving first: enlargement of Golgi complex as an early reaction to the primary physical intensity of brain trauma, and secondarily due to the anoxic-ischemic conditions of traumatized brain parenchyma, and second: loss of Golgi architecture, then progressively leading to fragmentation. Since in our studies we are working with cortical biopsies of living patients we can not establish by ethical reasons if disassembly and reassembly of Golgi fragments occur in nerve cells, as observed in normal mitotic cells [42]. Besides, we do not know weather disorganization of Golgi complex is a reversible process or a stage prior to reorganization, as described by Bainton et al. [43] in macrophages during phagocytosis of immobile immune complexes.

As above mentioned, the Golgi apparatus is altered by the secondary anoxic-ischemic process associated to the traumatic brain injuries. Our findings are supported by Petito [25] who showed transient swelling of Golgi apparatus in perineuronal oligodendroglial cells in areas of ischemic neuronal necrosis, and Petito and Pulsinelli [26] who described transformation of Golgi apparatus into large clusters of small vesicles without cisterns in cortical and striatal neurons following cerebral ischemia

The increased levels of calcium ion during ischemia seems to be responsible for brain edema not only due to due to opening of voltage-dependent calcium channels, but in addition by the release of calcium ion from their sites in the endoplasmic reticulum, particularly under the influence of IP3 and the ryanodine receptor, an endoplasmic reticulum calcium-release channel, that is opened by the elevated cytoplasmic calcium; hence giving rise to complex patterns of intracellular calcium signaling [39]. The calcium overload of brain parenchyma could also be responsible by the enlargement of Golgi smooth stacked cisternae and vesicles, as observed in our electron micrographs.

In brain edema and ischemia there is also an associated process of lipid peroxidation and membrane damage due to the release of arachidonic acid, free radicals [44-46], and nitric oxide [47]. This lipid peroxidation process explains the damage of smooth Golgi membranes. Rafols et al. [48], earlier stated that the areas of disturbed Golgi apparatus ultrastructure correspond to those areas that showed evidence of lipid peroxidation.

Clinical and neurosurgical implications of research findings

The findings herein described call the immediate neurosurgical treatment of patients with severe and complicated head injuries in order to avoid the secondary

ischemic process, which induce a catastrophic cascade of biochemical and molecular events upon plasma membrane [49] and nerve cell organelles, such as mitochondria [41], lysosomes [50], and the Golgi apparatus. The deleterious effects of brain trauma and ischemia on the synaptic region explain some clinical symptoms and neurological sequelae exhibited by the patients under study, as briefly described in Table 1.

CONCLUSIONS

The electron microscopic images suggest that the derangement of Golgi complex, the degeneration of myelinated axons, and consequently the interruption of axoplasmic flow in the traumatic brain injury primarily participate in the synaptic degeneration, and secondarily the ischemic process featured by calcium overload, glutamate and hemoglobin citotoxicity, and lipid peroxidation.

REFERENCES

[1]. Tani, E., Ametani, T., Nakano, K., Nishiura, M., Higashi, N, (1975) "Fenestrae in Golgi and endoplasmic reticulum cisternae of human brain tumours". *Acta Neuropathol.* 31:13-19.

[2]. Coxey, R.A., Pentchev, P.G., Campbell, G., Blanchette-Mackie, E.J. (1993) "Differential accumulation of cholesterol in Golgi compartments of normal and Niemann-Pick type C fibrobasts incubated with LDL: a cytochemical freeze-fracture study". *J. Lipid Res*. 176: 17-34.

[3]. Lucassen, P.J., Ravid, R., Gonatas, N.K., Swaab, D.F. (1993) "Activation of the human supraoptic and paraventricular nucleus neurons with aging and in Alzheimer's disease as judged from increasing size of the Golgi apparatus". *Brain Res*. 632 : 105-113.

[4]. Salehi A., Heyn S., Gonatas NK Swaab DF. (1995) "Decreased protein synthetic activity of the hypothalamic tuberomamillary nucleus in Alzheimer's disease as suggested by smaller Golgi apparatus". *Neurosci. Lett*. 193 : 29-32.

[5]. Xu, H., Greengard, P., Gandy, S. (1995) "Regulated formation of Golgi secretory vesicles containing Alzheimer beta-amyloid precursor protein". *J. Biol. Chem*. 270: 23243-23245.

[6]. Fujita Y., Ohama E., Takatama M., Al-Sarraj S., Okamoto K. (2006) "Fragmentation of Golgi apparatus of nigral neurons with alpha-synuclein-positive inclusions in patients with Parkinson's disease". *Acta Neuropathol*. 112 : 261-265.

[7]. Ghadially PN. (1991). "Golgi complex and secretory granules". In: *Ultrastructural Pathology of the Cell Matrix*. Ghadially, PN, ed. London: Butterworths. pp. 187-208,

[8]. Morré D. (1991) "Role of the Golgi apparatus in cellular pathology". *J .Elect. Microsc. Tech*. 17 : 200-221.

[9]. Castejón OJ. (1994) "Transmission electron microscope study of human hydrocephalic cerebral cortex". *J. Submicros. Cytol. Pathol*. 26: 29-39.

[10]. Fujita Y, Okamato K., Sukarai,A., Amari,M., Nakazato Y., Gonatas, N.K (1999) "Fragmentation of the Golgi apparatus of Betz cells in patients with amyotrophic lateral sclerosis". *J. Neurol. Sci*. 163: 81- 85.

[11]. Fujita Y., Okamoto K., Sakurai A., Kusaka H., Aizawa H., Mihara B., Gonatas, NK. (2002) "The Golgi apparatus is fragmented in spinal cord motor neurons of amyotrophic lateral sclerosis with basophilic inclusions". *Acta Neuropathol*. 103: 243-247.

[12]. Fujita Y., Okamoto K. (2005) "Golgi apparatus of the motor neurons in patients with amyotrophic lateral sclerosis in mice models of amyotrophic lateral sclerosis". *Neuropathology* 25 : 388-394.

[13]. Stieber A., Chen Y., Wei S., Mourelatos Z., Gonatas J., Okamoto K., Gonatas, NK. (1998) "The fragmented neuronal Golgi apparatus in amyotrophic lateral sclerosis includes the trans-Golgi-network: functional implications". *Acta Neuropathol.* 95 : 245-253.

[14]. Stieber A., Gonatas JO, Moore JS, Bantly A., Yim HS, Yim MB, Gonatas MK. (2004). "Disruption of the structure of Golgi apparatus and the function of the secretory pathway by mutants G93A & G85R of Cu, Zn superoxide dismutase (SOD1) of familial amyotrophic lateral sclerosis". *J. Neurol. Sci*. 219: 45-53.

[15]. Ramamohan PY, Gourie-Devi M., Nalini A., Shobha S., Ramamohan Y., Joshi P., Rahu TR. (2007) "Cerebrospinal fluid from amyotrophic lateral sclerosis patients causes fragmentation of the Golgi apparatus in the neonatal rat spinal cord". *Amyotroph. Lat. Scler*. 8:79-82.

[16]. Tougard C., Louvard D., Picart R., Tixier-Vidal, A. (1983) "The rough endoplasmic reticulum and the Golgi apparatus visualized using specific antibodies in normal and tumoral prolactin cells in culture". *J. Cell Biol.* 96:1197-1207.

[17]. Sakurai A., Okamoto K., Fujita Y., Nakazato,Y., Wakabayashi K., Takahashi H., Gonatas, NK. (2000) "Fragmentation of the Golgi apparatus of the balloned neurons in patients with corticobasal degeneratin and Creutzfeldt-Jakob disease*". Acta Neuropathol*. 100:270-274.

[18]. Takamine K., Okamoto K., Fujita Y., Sakurai A., Takatama, M. & Gonatas, N.K. (2000) "The involvement of the neuronal Golgi apparatus and trans-Golgi network in the human olivary hypertrophy". *J. Neurol. Sci*. 15:45-50.

[19]. Yaguchi M., Hashizume Y., Yoshida M., K. Gonatas N., Okamoto K. (2003) "Reduction of the size of the Golgi aparatus of spinal anterior horn cells in patients with X-linked spinal and bulbar muscular atrophy". *Amyotroph. Lat. Scler. Mot. Neuron Dis*. 4:17-21.

[20]. Yoshiyama Y., Zhan B., Bruce J., Trojanowski JQ, Lee VM. (2003) "Reduction of detyrosinated microtubules and Golgi fragmentation are linked to tau-induced degeneration in astrocytes". *J. Neurosci* 19:10662-10671.

[21]. Sahashi K., Ibi T., Ohno K., Sahashi K., Nakao N., Kondo H.. (2004) "Progressive myopathy with circulating autoantibody against giantin in the Golgi apparatus". *Neurology* 62:1891-1893.

[22]. Grieb P., Kryczka T., Fiedorowicz M., Frontczak-Baniewcz M., Walski, M. (2004) "Expansion of the Golgi apparatus in rat cerebral cortex following intracerebroventricular injectons of strptozotocin". *Acta Neurobiol*. 64: 481-489.

[23]. Ferrer I, Kapfhammer J.P., Hindelang C., Kemps S., Troffer-Charlier N., Broccoli W., Callyzot N., Mooyer P., Selhorst J.,Vrenken P., Wanders R.J., Mandel J.L., Pujol A. (2005) "Inactivation of peroxisomal ABCD2 transporter in the mouse leads to late-onset ataxia involving mitochondria, Golgi and endoplasmic reticulum damage". *Human Mol. Genet.* 14:3565-3577.

[24]. Gonatas NK, Stieber A., Gonatas, JO. (1986) "Fragmentation of the Golgi apparatus in neurodegenerative diseases and cell death". *J. Neurol Sci*. 246:21-30.

[25]. Petito CK. (2006) "Transformation of postischemic perineuronal glial cells. I. Electron microscopic studies". *J. Cerebr. Blood Flow Metabol*. 6:616-624.

[26]. Petito CK, Pulsinelli WA. (1984) "Sequential development of reversible and irreversible neuronal damage following cerebral ischemia". *J. Neuropathol. Exper. Neurol.* 43:141-53.

[27]. Tixier-Vidal A., Barret A., Picard R,. Mayau V., Vogt D., Wiedenmann B., Goud B. (1993) "The small GTP-binding protein, Rab6p, is associated with both Golgi and post-Golgi synaptophysin-containing membranes during synaptogenesis of hypothalamic neurons in culture". *J. Cell Sci*. 105:935-947.

[28]. Tixier-Vidal A.,-Faivre-Bauman A., Picard, R, Wiedenmann, B. (1988) "Immunoelectron microscopy localization of synaptophysin in a Golgi subcompartment of developing hypothalamic neurons". *Neuroscience* 26 : 847-861.

[29]. Toose SA, Hutnert WB. (1991) "Newly synthesized synaptophysin is transported to synaptic-like vesicles via constitutive secretory vesicles and the plasma membrane". *EMBO J*. 10:3589-3601.

[30]. Schmied R., Holtzman, E. (1989). Involvement of the Golgi apparatus in the sorting of material to opposite ends of frog retinal photoreceptors". *Neurobiology* 20:111-138.

[31]. Janetzko A., Zimmermann H., Wolknandt, W. (1989) "Intraneuronal distribution of a synaptic vesicle antibody binding sites at axonal membrane compartments and trans Golgi network and accumulation at nodes of Ranvier". *Neuroscience* 32:65-77.

[32]. Bauerfeind R., Huttner W. (1993) "Biogenesis of constitutive secretory vesicles, secretory granules, and synaptic vesicles". *Curr. Op. Cell Biol*. 5:628-635.

[33]. Nakata T., Terada S., Hirokawa N. (1998) "Visualization of the dynamics of synaptic vesicles and plasma membrana protein in living axons". *J. Cell Biol.* 140:659-674.

[34]. Dresbach T., Torres, N. Altrock, W.D.,Zamorano, P. Zuscratter W., Nawrotzki R., Ziv NE, Garner CC., Gundelfinder ED. (2006) "Assembly of active zone precursor vesicles: obligatory trafficking of presynaptic cytomatrix proteins Bassoon and Piccolo via trans-Golgi compartment". *J. Biol. Chem*. 281:6038-6047.

[35]. Morgans C., Brandstäter, JH. (2000) "SNAP-25 is present on the Golgi apparatus of retinal neurons". *Neuroreport* 11:85-88.

[36]. Hay JC. (1998) "Localization, dynamics, and protein interactions reveal distinct roles of ER and Golgi SNAREs". *J. Cell Biol*. 29:1489-1502.37

[37]. Castejón OJ.(1999) "Ultrastructural pathology of Golgi apparatus of nerve cells in human brain edema associated to brain congenital malformations, tumours and trauma". *J. Submicrosc, Cytol. Pathol.* 31: 203-213.

[38]. Gonatas NK., Stieber, A., Gonatas, JO. (2006) "Fragmentation of the Golgi apparatus in neurodegenerative diseases and cell death". *J. Neurol Sci.* 246:21-30.

[39]. Spuler A., Tan WKM., Mayer, F.B. (1996) "Molecular events in cerebral ischemia". In: Raffel, C., Hars, G.R., (Eds). *The Molecular Basis of Neurosurgical Diseases*. Baltimore: William and Wilkins. pp 248-269.

[40]. Castejón O.J., Acurero G. (2005) "Traumatic axolemmal and cytoskeletal derangement in myelinated axons of human oedematous cerebral cortex and loss of consciousness. An electron microscopic study using cortical biopsies". *J. Submicrosc. Cytol. Pathol.* 36: 285-293.

[41]. Castejón OJ., Castejón, HV. (2004) "Structural patterns of injured mitochondria in human oedematous cerebral cortex". *Brain Injury* 18:1107-1126.

[42]. Misteli T. (1996) "Molecular mechanisms in the disassembly and reassembly of the mammalian Golgi apparatus during M-phase". *FEBS Lett*. 389:66-69.

[43]. Bainton DF., Takemura R., Sternberg PE., Werb Z. (1989) "Rapid fragmentation and reorganization of Golgi membranes during frustrated phagocytosis of immobile immune complexes by macrophages". *Am. J. Pathol*. 134:115-126.

[44]. Ginsberg MD., Watson BD, Busto R. (1988) "Peroxidative damage to cell membranes following cerebral ischemia. A cause of ischemic brain injury". *Neurochem. Pathol*. 9 : 171- 173.

[45]. Mc Cord, JM. (1985) "Oxygen-derived free radicals in post-ischemic tissue injury". *New Eng. J. Med*. 312:159-163.

[46]. Siesjo BK., Gar CD., Bengtson, F. (1989) "Free radicals and brain damage". *Cerebrovasc. Brain Metabol. Rev*. 1:165-171.

[47]. Novicki, JP., Duval, D., Poignet, H. (1991) "Nitric oxide mediates neuronal death after focal cerebral ischemia in the mouse". *Eur. J. Pharmacol*. 204:339-340.

[48]. Rafols J.A., Daya A.M., O'Neil B.J., Krause G.S., Neumar R.W. ,White B.C. (1995) "Global brain ischemia and reperfusion: Golgi apparatus ultrastructure in neurons selectively vulnerable to death". *Acta Neurophathol*. 90 :17-30.

[49]. Castejón OJ. (2004) "Ultrastructural pathology of neuronal membranes in the oedematous cerebral cortex*". J. Submicrosc. Cytol. Pathol*. 36:167-179.

[50]. Castejón, O.J. (2004) "Lysosome abnmormalities and lipofucsin content of nerve cells of oedematous human cerebral cortex". *J. Submicrosc. Cytol. Pathol.* 36:263-271.