ALTERATIONS OF CELL MEMBRANES IN SCHIZOPHRENICS

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SUMMARY. In a previous work we made reference to the study of virus like particles observed with electron microscopic techniques in certain structures of the limbic system (amidgaline nucleus, hippocampus and the primary auditory cortex) of sixteen dead schizophrenic patients. As far as we know this is the only published paper about the ultrastructure of three of the four main regions of interest (ROIs) found in the brain of schizophrenic patients. In the last eleven years we have carried out studies of samples of blood of young schizophrenic patients during the critical stage of their disease observing that their platelets had changed in form, size and specific characteristics that let us differentiate patients of controls. In all these studies (nervous system and blood) we have observed deep alterations of cellular membranes with formation of big vacuoles, widened of the nuclear envelope in neurons, proliferation of cell membranes and in general a great relation of membranes structures to the origin of the virus particles.

INTRODUCTION. Current evidences related to morphometric and imaginologic studies have revealed a neuropathological involvement of the left temporolimbic-medial and superior temporal gyrus structures in schizophrenia that have been called “Regions of Interest (ROIs)” in the last studies should be to apply techniques at cellular level with more resolution power that could resolve the molecular biology and ultrastructure of the damaged cells. In agreement with Prof. Buchanan (1) related to the findings obtained in macroscopic imaging techniques: “these structural imaging studies can guide subsequent postmortem investigation and elucidate the ultrastructural changes underlying the observed structural alterations” Buchanan WR, Schizophrenia Bulletin 1998; 24: 501-18. Nevertheless there is practically a lack of ultrastructural research in these regions of interest. It has been in part due to the difficulties to obtain freshnes samples. In 1977 we started an electron microscope project (2) studying two limbic structures (amygdaline nucleus and hippocampus) and auditory cortex of the left cerebral hemisphere of young dead schizophrenic patients n=16 and controls n=10 observing three main alterations: nuclear bodies, membrane cell alterations and particles with viral morphology which react to herpes simplex hominis type I antibody (3)(REV NEUROL 2002; 33: 619-23). In the last eleven years we have carried out studies of samples of blood of young schizophrenic patients observing that their platelets have changed in form, size and specific characteristics that let us differentiate patients of controls (4). Some of these alterations are similar to those observed in the central nervous system and are compatible with: a) the viral hypothesis b) the herpes simplex hominis type I virus and c) with a deep alterations of the cellular membranes with formation of big vacuoles, widened of the nuclear envelope in neurons with particles and inclusion bodies, proliferation of cell membranes and in general a great relation of membranes structures to the viral like particles.

PATIENTS AND METHODS. Nervous system. Samples of the nervous system of a total of 16 deceased patients were studied, all with diagnostic criteria of schizophrenia for use in psychiatric research (5) and 10 controls deceased of causes not related with neurological neither infectious disease. The samples corresponded in all the studies to amygdala, hippocampus and primary auditory cortex of the left cerebral hemisphere. These were obtained before the first 24 hours after death. The first 24 hours postmortem, the electron microscopy was the following one: fixation in glutaraldehyde to 3,2% in buffer phosphate (Millonig) 0,15M pH 7,4, during one hour to 4 ºC and washing after that with buffer. Section of the samples and washed during the night in buffer to 4 ºC. Fixation with osmium tetroxide to 2% in buffer phosphate during one hour to 4 ºC and washed twice with buffer solution by five minutes each time to the same temperature. The dehydration was made in alcohol to the 50 and 70%, during five minutes in each phase, to the same temperature, and in alcohol absolute in three occasions, 10 minutes in each occasion, to ambient temperature. The inclusion was carried out in Epon 812. Fifty percent (50%) of the patients had less than forty years (Table). All were under neuroleptic treatment. Blood. Samples of venous blood (3ml) was drawn from a peripheral vein of a total of 70 schizophrenic patients (50 Cubans and 20 Spaniards) with DSM-IV diagnostic criteria (6) and different clinical forms and 100 controls (50 Cubans and 50 Spaniards). The samples were obtained by means of informed consent, according to the ethical approaches for research and with the approval of the Committee of Ethics of each institution. The samples of patient and Cuban controls proceeded from the city of Havana. The one of patient and Spanish controls from the city of Malaga, Andalusia. All the samples were obtained in test tubes with EDTA type potassium anticoagulant. The ages of all the patients and controls were between 15 and 35 years. Both sexes were represented in a proportional form. To each patient and control was carried out a questionnaire to obtain data related with name, age, date of birth, family antecedents of psychosis; in the case of the patients: psychiatric symptoms present, time of evolution of the illness, age, sex, race, received treatment, as well as frequency and duration of the crises. The blood samples were centrifuged at 1.000 rev/min during 10 minutes; obtained the platelet-rich plasma it was centrifuged later on at 3.000 rev/min during 10 minutes. Then they were studied by means of the following
ultramicroscopic techniques: fixation in buffer phosphate containing 3% glutaraldehyde (Millonig) 0.15M pH 7.4, during one hour at 4 °C and washed with buffer, later on. Section of the samples and washed during the night in buffer at 4 °C. Then fixation with 2% osmium tetroxide in buffer phosphate during one hour at 4 °C and washed twice with buffer solution by five minutes every time at the same temperature. The dehydration was made in 50 and 70% alcohol, during five minutes in each phase, at the same temperature, and in absolute alcohol in three occasions, 10 minutes in each occasion, at ambient temperature. The inclusion was carried out in araldite.

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RESULTS.

1. Widening of the nuclear envelope in neurons frequently observed in samples from the amygdala and the auditory cortex.
2. Presence of fibrillar material within this widened nuclear envelope.
3. Presence of inclusion bodies. Fig. 1
4. Virus like particles within the widened nuclear envelope. Fig. 2
5. Nuclear bodies and intranuclear filamentous inclusions.
6. Presence of big vacuoles in platelets with fibrillar material and virus like particles within these vacuoles. Fig. 3a 3b
7. Presence of herpes simplex hominis type I antigen in particles related to membranes structures. Fig. 4a 4b
**Fig. 1**

Widening between the two components of the nuclear envelope. Presence of inclusion bodies (arrows) in the widened nuclear envelope. Sulfurphosphate patient. 30,000X.

**Fig. 2**

Virus-like herpes particles within the widened nuclear envelope of a brain sample from the amygdala of a schizophrenia patient. The particles (arrows) resemble the form that herpes virus use to leave the cell with a budding particle above (double arrow).
Fig. 3a Vacuole at the end of a arrow) with an incomplete sph (arrow head) and fibrillar mater.
Fig. 3b Part vacuole. The vacuole contains glycogen granules budding from microsomes.

Fig. 4a Platelet (plaq) of a schizophrenic critical stage. Size=100 nm.

Platelet

part

plaq

Platelet with anti-herpes hominis type I surrounding the filopodia. Negative electron microscopy method.
DISCUSSION. In a review of the indexed publications giving the key words schizophrenia, temporal lobe and electron microscopy we will find that only exist in the medical literature 6 works related to this theme of those in only one the study is made of three of the main four regions of interest (ROIs) observed in the brain in schizophrenia: amygdala, hippocampus and temporal transverse gyrus of Heschl (41 area of Brodmann)(3). The rest of the studies carried out by other authors, in only three, in fact relevant with this topic, the studies are centered in other areas of the temporal cortex (temporo-polar cortex, 38 area, superior temporal cortex not auditory) and they were directed toward studies of the microglia, the sinapsis and levels of mRNA encoding sinaptic vesicles, being studied in occasions only 1 patient or patients with very advanced ages (7-9). In our works, that we began in 1977 (2)(3) we selected the brain samples from the left cerebral hemisphere, in fact where more affected the alterations have been later reported mainly by imaginologic and morphometric methods (10,11) and 50% of the patient studied had less than 40 years old. Since that date we have made successive publications in this sense, as we were observing new findings. Among them it is worth while to highlight the alterations at the cellular membranes level and their relationship with the particles similar to virus, later on identified by immuno-electron microscopy like herpes virus hominis type 1(12).

In relation to the ultrastructural studies of platelets in the peripheral blood of schizophrenic patients only appear in the medical literature, at least indexed, three works (13,14,3), one of those we carried out starting from the observation of a precipitation reaction when the plasma and the cerebrospinal fluid of the own patient were put on direct contact (15,16). The membrane alterations found in platelets of schizophrenic patients look like the alterations found in the central nervous system. Big vacuoles originates of the same ones with presence of inclusion bodies or particles like virus. The immuno-electron microscope technique used in these studies on platelets has allowed to demonstrate the presence of herpes simplex hominis type 1 virus antigen, the same one observed in the central nervous system. However the few cases studied with this technique don't allow us to reach to definitive conclusions Fig. 4.

Unless our findings can be confirmed in a larger number of schizophrenic patients, one can not rule out the possibility of a separate neuropathological condition, unrelated to schizophrenia. Could the patient have had an unrecognized HERPES virus infection? Specially the fact, that the material was obtained from the temporal lobe justifies the question. Is the outer nuclear membrane blistered out, could be ante-mortem or post-mortem? As the particles observed within the nuclear envelope?

It was observed in our studies that the widening of the nuclear envelope was more frequently seen in the material obtained from the amygdala and the auditory cortex; if it concerned related to the post-mortem alterations, the same changes must have been found too in the samples obtained from the hippocampus, if it was connected to post-mortem structural changes. Furthermore the presence of fine fibrillar material filling all the widened space between the two components of the envelope, was a constant finding, which agrees with a real alteration and not with an artefact, as the last one should be variable. In some occasions inclusion bodies with a definite structure have been seen together with this fibrillar material. These inclusion bodies haven't been reported before and their finding and the finding of the fibrillar material in the widened envelope put far away the possibility of them, being the result of an artefact and outlines their
possible relation to specific alterations in specific structures in this disease. The fact that these alterations are seen in structures of the limbic system and auditory cortex related for the time being to schizophrenia make us think even more that it is not a case of post-mortem alterations.

What could be the significance of these alterations in the mentioned disease? The inclusion bodies have been related in general to viral diseases (17,18). We have observed within the altered nuclear envelope inclusion bodies which due to their great dimension can not be occasionally considered as mature viral particles neither could be the fibrillar material, unless the first were viral conglomerations and the second ones were incomplete viral forms. However, we have occasionally seen within the nuclear envelope particles which by size and general configuration had been considered as possible viral particles. The fact that they have the range of 60 to 100 nm outlines a new question: its possible relation to our findings about the inoculation of chicken embryos with CSF from schizophrenics (19). The isolated viral particles seen by us had the same range of size as the ones we observed in the widened nuclear envelope. It was confirmed by immuno-electron microscopy identification as herpes virus type I in schizophrenic human brains, brains of fetuses from schizophrenic mothers and brains from experimental inoculation of chicken embryos. It is well known that there is a relation between herpes virus and the membrane structures of the cells specially related to the nuclear envelope and the presence of intranuclear membrane-bound, dense, rounded inclusions, 100 to 500 nm in diameter in human fibroblasts infected with herpes simplex (20). Our findings of particles related to the nuclear envelope make us think in the already mentioned question if there is some relation between schizophrenia and herpes virus diseases due to its clinical form of evolution, its epidemiological aspect, the organic and chemical alterations, the presence of cytopathic effect in tissue cultures and experimental animals (21-30) and more recently the presence of viral antibodies in the blood to herpes virus hominis type I in schizophrenia and bipolar disorders (31,32). In a meta-analysis of Profs. Yolken and Torrey about "Viruses, Schizophrenia and Bipolar Disorder" (33) they claimed: "Direct testing of a viral hypothesis of serious mental illnesses began in the 1950s. Utilizing the technology that was then available, Morozov (34) and his colleagues in the Soviet Union claimed to have microscopically seen virus-like corpuscles in the CSF and nasal secretions of many patients with schizophrenia. In Italy, Mastrogiovanni and Scarlato (35) inoculated CSF from patients with schizophrenia into chicken embryos and also claimed to have microscopically visualized virus-like particles. Since that time, the only researchers who have claimed to have found virus particles in patients with serious mental illnesses have been Castillo and his colleagues in Havana, Cuba. They have described intracytoplasmic encapsulated structures similar to herpesviruses in freshly obtained postmortem brain tissue from patients with schizophrenia and also in brain tissue from aborted fetuses from mothers with schizophrenia" (36,37). Torrey EF, Yolken R. Viruses, Schizophrenia and Bipolar Disorders. Journal Microbiology Review, Jan 1995.
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