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BCRP Expression with Lipofuscin Accumulation in Abnormal Neurons from a Child with Transmantle Cortical Dysplasia (TMCD) and Refractory Epilepsy

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ABSTRACT

Cerebral cortical development's malformations, including the transmantel cortical dysplasia (TMCD), have been associated with refractory epilepsy (RE). Several ABC-transporters as "P-glycoprotein (P-gp), Multidrug resistance proteins (MRP-1) and breast Cancer Resistant Protein (BCRP)" are up-regulated in human epileptogenic brain lesions of RE, however they have not been explored in Transmantle cortical dysplasia (TMCD).

We describe a 13 years old boy with Refractory Epilepsy (RE) and abnormal Magneic Resonance Image (MRI) (T1, FLAIR and T2) compatible with TMCD. Clinical follow-up, images and pathologic studies were developed by routinely methods. Epilepsy surgical treatment included total lesion resection with complete seizures remission at date.

Deeper brain areas related with images findings, showed features of TMCD with abnormal ballooned neurons with high accumulation of PAS+, sudanophilic and autoflourescent lipopigment (LP). Immunohistochemistry, using primary monoclonal antibodies for P-gp, MVP and BCRP proteins, showed high expression of BCRP in several ballooned-LP+ cells. In contrast, P-gp and MVP were negative and MRP-1 has not been investigated.

The links of BCRP with LP and AEDs are not known, however, the expression of BCRP in these LP+ ballooned neurons from the epileptogenic brain area, with P-gp/MVP negative results, suggest that BCRP could be associated to refractory epileptic phenotype.

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Introduction

Several malformations of cortical development have been described associated with refractory epilepsy and developmental delay. The main malformations identified were heterotopic gray matter, cortical tubers, focal cortical dysplasia, polymicrogyria, agyria-pachygyria, schizencephaly/cleft, transmantle dysplasia, and hemimegalencephaly [1, 2].

The transmantle cortical dysplasia (TMCD) was reported as a specific anomaly resulting from abnormal stem cell development, representing 5% of the main malformations identified [3,4]. TMCD was first described in 18 patients younger than 20 years with epilepsy or fixed neurologic deficits that presented MRI signal abnormalities extending from the cortex to the superolateral wall of the lateral ventricle. The histological features of their brain biopsies are characterized by cortical disorganization, neuronal cytomegaly, balloon cells, indistinct cortical gray matter-white matter junctions, and variable accompanying astrogliosis. A specific anomaly resulting from abnormal stem cell development was proposedand [3].

Similar malformations of cortical development have been also described as additional lesions in tuberous sclerosis complex [5,6].

Two proteins associated with multidrug resistance in cancer, P-glycoprotein (P-gp) and multidrug resistance-associated protein 1 (encoded by ABCB1 and ABCC1 genes respectively), are up-regulated in human epileptogenic pathologies and the molecular basis of drug resistance in epilepsy is being intensively explored in experimental epilepsy models [7-21].

Another transporter named as "brain multidrug resistance protein" (BMDP) has been discovered at the porcine blood brain barrier (BBB) and was shown to be highly homologous to the human breast cancer resistance protein (BCRP), which is an other ABC transporter that confer multidrug resistance phenotype to the expressive cells [22-25]. However at date, all studies comparing BCRP expression in control and epileptic human brain tissue demonstrated the constitutive expression of BCRP in the brain capillary endothelium, but these data do not show differences in BCRP expression levels between the groups. Due to the current lack of evidence on BCRP overexpression in human epileptic brain tissue, BCRP is unlikely a major player in ASD resistance as proposed by the transporter hypothesis [26]. Here we describe at the first time the high BCRP expression in abnormal neurons loaded with lipofuscin from epileptogenic brain area. in a pediatric case of refractory epilepsy due TMCD.

Case Report

We describe a 13 years old boy with right focal clonic seizures (upper members and half body) from 5 month of life, without others personal or familial antecedents. Initial neurological examination, laboratory studies, EEG and CT scans were normal. Isolated crisis persisted thorough 8 years with normal IQ, normal intercritic EEG or left focal spikes. After this age, the number and intensity of seizures were increased without control with different antiepileptic drug schedules, and an other CT scan and MRI without abnormalities. (not shown)

Actually, at 13 years old, he present a chronic story of daily multiple crisis, light left hemiparesia and IQ = 75.

A recent MRI study indicated the engrossment of left frontal cortex, associated to an brain area with light signals changes related to gray matter, extended to subjacent left ventricle (hyper-intense in T1 and I/R, hypo-intense in FLAIR and isoi-ntense in T2), compatible with diagnosis of TMCD (Figure 1).

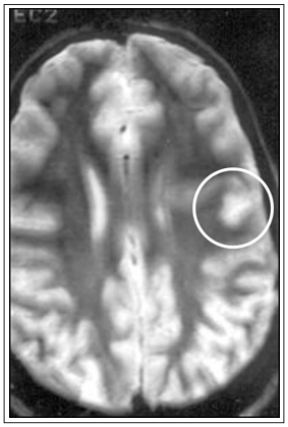

Figure 1: RMN -T2 showing the epileptogenic TMCD

The surgical resection of the brain epileptogenic lesion areas were developed and now, the patient remains without crisis since one year ago.

The brain material was examined by routinely histochemical methods, electronic microscopy examination, and used to investigate the expression of P-gp, MVP-1 and BCRP transporters.

Methods

Brain Tissue Samples

Surgical specimens of brain tissues were selected from the tissue collection of the Pathology Laboratory of the Garrahan Children's Hospital of Buenos Aires. Patient had been surgically treated for intractable epilepsy, and brain samples were surplus to diagnostic requirements.

Morphological Analysis

Brain tissue was fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin, Nissl, Luxol-Fast-Blue with PAS and Sudan Black methods, for morphological analysis.

Immunohistochemistry Methods

• Drug Transporters: The monoclonal antibodies and dilutions were used as follows: P-gp (1:50, clone JSB-1; Novocastra, Newcastle Upon Tyne, UK), MVP (1:50, Signet-Dedham, MA), and BCRP (1:50, Kamiya Biomed.Co.- Seattle). MRP-1 was not investigated.

• Secondary polyclonal antibody was performed with a Streptovidin immunoperoxidase kit, according to the protocol recommended by the manufacturer (Biogenix, San Ramón, CA, USA).

Results

A-Pathological Findings

Biopsy evidenced normal left frontal cortex and a deep abnormal area with totally ballooned neurons loaded with PAS+ sudanofilic and autoflourescent compound, corresponding to lipopigment likes to neuronal ceroid lipofiscinosis. (Figure 2a)

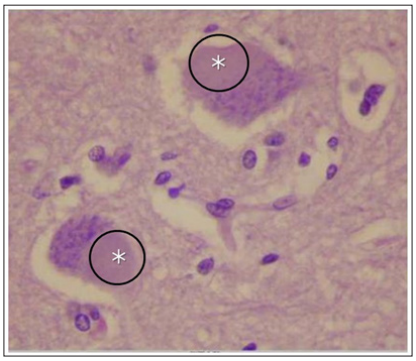

Figure 2: Hematoxilin-Eosine staining: Ballooned neurons with lipofuscin-like lipopigment accumulation (*)

Electronic Microscopy

Granular-dense and vacuolar electron-clear material with characteristics of classic lipofucsine are observed (Figure 3 Magnifications: a- 4000X, b-22000X)

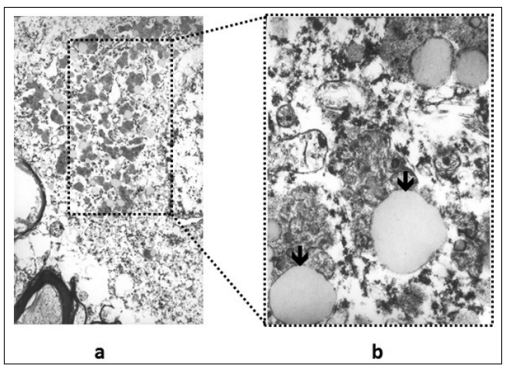

Figure 3: vacuoles are indicated by a red arrow (b)

B-Inmunohistochemistry

Drug Transporters

P-glycoprotein (P-gp) was located in the luminal membrane of brain capillary endothelial cells without immunostaing differential

pattern compared with normal brain areas. Similarly, BCRP was highly expressed in BBB from both normal and pathological areas.

However, in brain parenchyma cells from epileptogenic lesion, P-gp and MVP were not detected, but strikingly BCRP was highly expressed in the abnormal ballooned neurons, with a particular polarized distribution in opposite side of lipopigment accumulation (Figure 4).

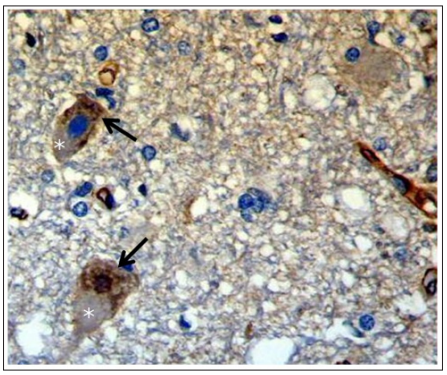

Figure 4: BCRP IHC: Ballooned neurons presenting polarized negative areas with lipid accumulation (*) and positive immunostaining for BCRP in the opposite site (➔)

The lipofuscin-like lipopigment accumulation was restricted to abnormal brain area of the mantle dysplasia without diffuse distribution as observed in neuronal ceroid lipofucsinosis or physiologic aging, and unusually it was accumulated in abnormal neurons.

Discussion

The peculiar cytological features of the ballooned abnormal neurons observed in the specimens from the epileptogenic brain lesion of our patient, are not compatible with previously described in ballooned cells from Taylor´s cortical dysplasia, tuberous sclerosis, or the findings observed in aging and it was not according with previously described features of childhood neuronal ceroid-lipofucsinosis in Argentina [27]. Intensive lipopigment accumulation was restricted to the abnormal cells from the lesion´s area, without diffuse and/or extensive distributive pattern as described in others conditions with ceroid lipofucsin accumulation [28,30].

The clinical and brain images features, correlated with a cortical developmental disease compatible with a trasnmantle cortical dysplasia and refractory epilepsy [3,4].

The seizures abrogates with the surgical treatment by resection of the abnormal brain area, indicates that the particular morphological features correlated with the epileptogenic activity of the lesion.

At our knowledge, the blood-brain barrier (BBB) plays the predominant role in controlling the passage of endogenous and xenobiotic substances between the circulating blood and the extracellular fluid environment of the brain. So far, the multidrug resistance in epilepsy has been almost exclusively attributed to MDR-1 gene encoded P-glycoprotein (P-gp), the most prominent member of the ATP-binding cassette (ABC) transporter family, linked to higher expression in the luminal membrane of brain capillary endothelial cells and also expressed in brain parenchyma cells including neurons and astrocytes [7-21].

More recently, a called “brain multidrug resistance protein” (BMDP) has been discovered at the porcine BBB. Phylogenetic analysis and multiple sequence alignment showed that porcine BMDP is most related to the human and mouse breast cancer resistance protein (BCRP) [22,31].

It was demonstrated by Immunofluorescence confocal microscopy that BCRP is normally located at the blood-brain barrier, mainly at the luminal surface of microvessel endothelium resembling that of P-gp. Because bouth transporters have several common substrates, BCRP may give an additional barrier to drug access to the brain [32,33]. However, at date the relationship between BCRP and AEDs still remains to be clarified.

It was reported that BCRP is expressed ubiquitously in brain capillary endothelium in patients with RE, but the authors concluded that there was no qualitative up-regulation of this transporter [34].

More recently, Vogelgesang S et al have been demonstrated that BCRP was highly expressed in vascular endothelial cells (VEC) of BBB as well as in astrocytes from brain specimens of patients with dysembrioplastic neuroepithelial tumors and RE [35].

In our patient, the P-gp and MVP were negative in brain parenchyma cells, and we can’t study MRP-1 expression, however BCRP was strongly immunoreactive not only in VEC of BBB, but also in several abnormal neurons with high lipid accumulation, being the first observation of BCRP expressed in LP+ abnormal neurons associated with both epileptigenic and refractory phenotypes.

The relationship between the ABC transporter BCRP and LP has not been studied. In this regard, it important to notice that mutations on ABCA4 gene (an other member of ABC transporter family, also known as ABCR) has been demonstrated related with a Rod photoreceptor retinoid transport alteration and over 300 mutations in this ABCR gene have been associated with a variety of clinically distinct autosomal recessive retinal degenerative diseases, including Stargardt macular dystrophy, fundus flavimaculatus, cone-rod dystrophy, and retinitis pigmentosa , characterized by lipofuscin accumulation [36-40]. The lipofuscin accumulation is a features of ABCR gene mutation in Stargardt and also in the Age-Related Macular Degeneration diseases and it is coincident with the descriptions observed in the ABCR nockout mice, suggesting that similar mechanisms could be present in our case of the TMCD [41].

Our results suggest that BCRP expression in ballooned neurons can’t protects this abnormal cells against the lipofuscine accumulation. We don’t know if our patient have any ABCR mutation, or if he have any BCRP polymorphism associated with this dysfunctional lipid transport. However, it has been suggested that humans or animals with low or absent BCRP activity may be at increased risk for developing protoporphyria and diet-dependent phototoxicity. Interestingly, lipofiscine observed in our case was a fluorescent pigment, and the primary pathologic defect in Stargardt’s disease is accumulation of “toxic lipofuscin pigments” such as N-retinylidene-N-retinylethano-lamine (A2E) in cells of the retinal pigment epithelium. This accumulation appears to be responsible for the photoreceptor death and severe visual loss in Stargardt’s patients. Recently it has probed that treatment with isotretinoin may inhibit LP accumulation and thus delay the onset of visual loss in Stargardt’s patients, perhaps giving an alternative treatment in TMCD cases with lipofiscin accumulation as described here [42].

In summary, our case of TMCD showed a particular pattern of immunohistochemistry on brain parenchyma cells, characterized

by non-detectable P-gp/MVP proteins, but high BCRP expression in LP+ abnormal neurons from epileptogenic brain area.

These results suggest that BCRP could play a role in the development of refractory epilepsy phenotype.

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