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Extensive apoptosis in a case of intractable infantile status epilepticus

Koshi A. Cherian^{a,c,d,*}, Karen Weidenheim^{a,e,g}, Alan D. Legatt^{a,b,d}, Keivan Shifteh^f, I. Richmond Abbott^g, and Solomon L. Moshé^{a,b,c,d}

^aSaul R. Korey Department of Neurology, Albert Einstein College of Medicine and Montefiore Medical Center, United States

^bDominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine and Montefiore Medical Center, United States

^cDepartment of Pediatrics, Albert Einstein College of Medicine and Montefiore Medical Center, United States

^dMontefiore/Einstein Epilepsy Management Center, Albert Einstein College of Medicine and Montefiore Medical Center, United States

^eDepartment of Pathology (Neuropathology), Albert Einstein College of Medicine and Montefiore Medical Center, United States

^fDepartment of Radiology (Neuroradiology), Albert Einstein College of Medicine and Montefiore Medical Center, United States

^gLeo M. Davidoff Department of Neurological Surgery, Albert Einstein College of Medicine and Montefiore Medical Center, United States

Summary

A previously healthy 8½-month-old girl underwent epilepsy surgery for intractable focal seizures with secondary generalization that progressed to status epilepticus. The major neuropathologic finding was extensive apoptosis. Investigations did not reveal any etiology for the apoptosis or the seizures. This is the first report of apoptosis, without necrosis, in association with intractable status epilepticus in the developing human brain. The findings suggest that new treatment strategies targeted to prevent apoptosis may be useful in children with prolonged status epilepticus.

Keywords

Status epilepticus; Infant; Apoptosis; Epilepsy surgery

Introduction

Seizure disorders in infancy are commonly caused by acquired lesions such as infections or neoplasms, by developmental aberrations such as cortical dysplasia, or by genetic disorders such as lysosomal storage diseases. In most cases, a cause is identified after clinical, laboratory

and neuroimaging study. Herein, we report a case of intractable epilepsy in a previously healthy infant in which the major neuropathologic finding was extensive apoptosis, with only minor, microscopic cortical microdysplasia, which was insufficient to cause extensive cell death and seizures. This case is unusual because no underlying infectious, neoplastic or developmental cause could be identified in the epileptogenic cortex, to account for the extensive apoptosis.

Case report

At the age of 8½ months, a previously healthy girl had several episodes of vomiting over several days and then developed episodes of jerking movements of the left side of the body, predominantly the left leg, that increased in duration to more than 30 min each (Day 0). This was accompanied by an altered mental status. The jerking movements were initially controlled with lorazepam and phenobarbital but they recurred 2 days later, now involving the trunk and the left arm and leg. On Day 3, she became febrile and had repeated episodes of generalized tonic—clonic seizures. She remained in status epilepticus despite treatment with phenobarbital, fosphenytoin, valproate, intravenous pyridoxine (two doses, 100 mg each), immunoglobulin G, methylprednisolone and the induction of pentobarbital coma.

She had been born at term to nonconsanguineous parents and her developmental milestones had been normal. There was no family history of seizures or mental retardation. The mother denied any history of drug use or intoxication. The CSF protein and glucose were normal. The diagnostic workup (done on Day 4 and Day 7) including cerebrospinal fluid lactate, serum pyruvate, serum amino acids, urine organic acids, blood lead, copper, and mercury levels, herpes simplex virus polymerase chain reaction (in both CSF samples) and encephalitis panel, bartonella, leptospira, mycoplasma, and rabies titers was negative. Epstein Barr virus IgG was positive at 1:320 but IgM and early antigen were negative; the IgG may be positive due to the immunoglobulin G she had received, and does not suggest an active infection.

The initial EEG (Fig. 1A) consisted of a mixture of theta and delta activity intermixed with faster frequencies (Day 9). There were frequent sharp waves over the right central region. Continuous scalp video EEG monitoring showed multiple electroclinical seizures that began in the right central head region, and spread to both hemispheres. As the pentobarbital infusion was increased, the clinical manifestations disappeared but the patient continued to have frequent electrographic seizures (Fig. 1B), lasting less than 5 min. Magnetic resonance imaging (MRI, Fig. 2) on Day 22 showed an area of abnormality in the right parietal region with poor gray—white distinction and increased signal in the subcortical white matter, suggestive of cortical dysplasia.

Because of the refractory focal seizures and the focal findings on EEG and MRI, a right parietal craniotomy was performed on Day 23. Intraoperative electrocorticography showed very frequent spikes, maximal at an electrode near the center of the MRI abnormality. A resection was performed, and was extended anteriorly twice because of very frequent spiking at the anterior margins. Post resection, electrocorticography following showed persistent frequent spiking at the anterior margin, but the resection was not extended further due to concerns about encroaching on the central sulcus.

Histopathologic sections of neocortex and white matter showed an estimated 50% loss of neurons from the cortical ribbon (Fig. 3A). Approximately 40% of the remaining cells had the dense pink cytoplasm and nuclear karyorrhexis indicative of apoptosis (Fig. 3B). The apoptotic neurons were most numerous in the second and fourth cortical layers. A few dysmorphic neurons were present in cortical layer I (Fig. 3C), suggestive of microdysgenesis. Immunohistochemical studies showed diffuse astroglial staining (Fig. 3D), and activation of microglia. There were no microglial nodules, viral inclusion bodies, neuronophagia, or other

evidence of encephalitis. Special stains for bacteria and fungi were negative. Immunohistochemical staining using antibodies against herpes virus, Epstein Barr virus and cytomegalovirus were also negative.

The seizures recurred on Day 26, and surgery with electrocorticography was performed on Day 28. An evolving electrographic seizure was captured, originating at the anterior/superior margin of the previous resection (Fig. 4). This area was resected, along with additional areas of cerebral cortex where electrocorticography showed very frequent spiking, including runs of repetitive spikes. Histopathologic analysis of the tissue removed during the second operation showed a well-preserved cortical ribbon (Fig. 3E; compare to Fig. 3A) and only scattered apoptotic cells (Fig. 3F; compare to Fig. 3B). Gliosis was again seen but there was no evidence of encephalitis. MRI performed one day after the second surgery (Day 29) showed multiple areas of recent infarction in the right frontal and parietal lobes with no evidence of atrophy or hippocampal sclerosis.

Unfortunately the seizures persisted and the child was left significantly impaired. At last follow-up, she was blind, nonverbal, unable to sit without support, and fed by a gastrostomy tube.

Discussion

We report herein an unusual case of sudden onset status epilepticus in a previously healthy 8½-month-old girl. The etiology for the occurrence of status epilepticus was not fully elucidated even after the two cortical resections from the areas with the most active epileptic discharges. The neuropathology in the area of the most pronounced neuroimaging and electrophysiologic abnormalities showed marked neuronal loss with extensive ongoing apoptosis that diminished in the interval between the two surgeries. No neuronal necrosis was seen. There was extensive gliosis and only minimal cortical microdysplasia. The marked neuronal loss most likely reflects the endstage of the apoptotic process in this region. The apoptosis and neuronal loss were both less prominent in the adjacent tissue, which was obtained at the time of the second resection. What does this mean?

Apoptosis is the morphological term used to denote the physiologic process by which metazoan organisms remove unwanted cells (Uren and Vaux, 1996; Zhang et al., 2004/2005). It is characterized by cell shrinkage, condensation of the chromatin to the margins of the nuclear membrane, nuclear breakdown, membrane blebbing and eventual fragmentation of the cell into apoptotic bodies that are rapidly engulfed and degraded by neighboring cells or phagocytes, usually without disturbing the tissue architecture (Uren and Vaux, 1996; Zhang et al., 2004/2005; Ellis and Horvitz, 1986). In contrast to apoptosis, necrosis occurs when the cell is killed following damage to a vital cellular process (Uren and Vaux, 1996; Zhang et al., 2004/2005; Ellis and Horvitz, 1986). Necrotic cellular debris are cleared by an inflammatory infiltrate of phagocytic cells and there is often associated scarring (Uren and Vaux, 1996; Zhang et al., 2004/2005; Ellis and Horvitz, 1986).

Dysregulation of apoptosis is implicated in a variety of human diseases such as HIV infections, strokes, neurodegenerative disorders, traumatic brain injury, spinal cord injuries and meningoencephalitis (Ameisen and Capron, 1991; Antoni et al., 1995; Finkel et al., 1995; Liou et al., 2003; Crowe et al., 1997; McManus and Buchan, 2000; Rink et al., 1995). None of these conditions were present in our case.

Many drugs and toxins have been shown to induce apoptosis in experimental studies. Compounds used as sedatives, anesthetics or antiepileptic drugs (AEDs) trigger widespread apoptotic neurodegeneration throughout the developing brain when administered to immature rodents during the period of the brain growth spurt (Kaindl et al., 2006; Bittigau et al., 2002;

Ikonomidou et al., 1999; Ikonomidou et al., 2000; Jevtovic-Todorovic et al., 2003). Such compounds include drugs which alter physiologic synaptic activity i.e. antagonists of NMDA receptors (ketamine, nitrous oxide), agonists of GABA subtype A receptors (barbiturates, benzodiazepines, propofol) and sodium channel blockers (phenytoin, valproic acid) (Kaindl et al., 2006; Bittigau et al., 2002; Ikonomidou et al., 1999, 2000; Jevtovic-Todorovic et al., 2003). It seems unlikely that the apoptosis in our patient was related to the aggressive AED treatments, because it was not uniform in the brain and also, it was reduced in the second specimen, even though the AED treatment continued. It is worth noting that the brain growth spurt occurs at different times relative to birth in different species. While in rats and mice it occurs postnatally, in humans it extends from the sixth month of gestation to several years after birth (Dobbing et al., 1979).

In experimental studies, status epilepticus can induce apoptosis, especially in adult animals (Haut et al., 2004). However, status epilepticus induced in the immature brain is not often associated with apoptosis. Indeed, developing neurons are less vulnerable, in terms of seizure-induced neuronal damage and cell loss, than are adult neurons. There are very few reports that a mild degree of apoptosis may occur in the developing brain following status epilepticus (for review please see, Haut et al., 2004; Ben-Ari and Holmes, 2006).

The cause of the catastrophic course of the cases reported by Mikaeloff et al. (2006) and Baxter et al. (2003) under various names... has not been elucidated. For example, Baxter et al. (2003) described 6 infants and children with sudden onset of focal or multifocal seizures building to refractory status epilepticus resulting in death or severe neurological disability with cortical atrophy. However, the neuropathological findings were nonspecific. There was no apoptosis but this lack may be a reflection of when the tissue was examined as apoptosis might have diminished with time as it was the case in our patient. It is possible that a common feature in all these case is the sudden onset of apoptotic cell loss leading to status epilepticus and dire sequelae.

This report documents the occurrence of florid apoptosis, without necrosis, in association with refractory status epilepticus in the developing human brain. It is unclear whether the apoptosis was a cause or an effect of the prolonged seizures or whether, as seems more likely, both were caused by an unknown insult in this patient. Clearly, there is a relationship between them, as the apoptosis was most prominent in the region of the brain that was most abnormal electrophysiologically and on neuroimaging. Consideration should be given to the institution of new treatment strategies targeted to prevent apoptosis in children with status epilepticus of unknown etiology.

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Figure 1.

(A) Initial scalp EEG recording, showing frequent sharp waves in the right central region (asterisks). There were occasional sharp waves at Cz, often associated with the right central sharp waves, with a phase lag (see vertical red line), but also occurring independently (longitudinal bipolar montage). (B) Scalp EEG recorded during continuous video-EEG monitoring, showing a focal seizure discharge over the right parasagittal central region with subsequent spread (referential montage, linked O1—O2 reference; the time scale is compressed by a factor of 2 compared to figure A).

Figure 2. MRI (A) coronal T1 weighted and (B) axial T2 weighted images demonstrated abnormally thickened cortex in the high right parietal lobe (arrows).

Figure 3.

(A) A low power view of the cortical gray ribbon from the first resection shows marked decrease (estimated at 50%) of brown staining neurons in the cortical lamina. The molecular layer is seen on the right marked by short arrows. NeuN immunohistochemistry, original magnification $\times 20$. (B) Approximately 40% of the remaining cells showed the dense brightly eosinophilic cytoplasm and nuclear karyorrhexis of apoptotic cells (arrows). Hematoxylin and eosin, original magnification $\times 1000$. (C) Neurons were easily found in the molecular layer (short arrows) consistent with microdysgenesis. NeuN immunohistochemistry, original magnification $\times 200$. (D) There was a moderate gliosis, as shown by the numerous brown astrocytes and their processes, throughout the specimen. Immunohistochemical method for glial fibrillary acidic protein, an astrocyte marker. Original magnification $\times 200$. (E) The neocortical ribbon was much better preserved in the material from the second resection as seen in this section stained using NeuN immunohistochemistry. The molecular layer is at right, marked with short arrows. Compare to panel A from the first resection, at the same magnification. Original magnification $\times 20$. (F) Only scattered apoptotic cells (arrows) were present. Compare this panel to panel B from the original resection. Hematoxylin and eosin, original magnification $\times 1000$.

Figure 4.

(A) Electroecorticogram recorded during surgery on Day 28, from a 4×5 electrode grid (with 1 cm inter-electrode distance) placed at the superior/anterior margin of the previous resection. Channels that were noisy because of poor electrode contact are omitted. The recordings were referred to a needle electrode in the scalp (“S”). Recurrent spikes that were largest at electrodes A3 and A4 became more frequent and developed into an evolving electrographic seizure. The seizure was terminated 80s after onset by irrigation of the cortical surface with cold saline. (B) Diagram of the approximate position of the electrode grid and its relationship to the prior resection (cross-hatched area).