# LIGHT AT THE END OF THE "TUNEL"? ROLE OF CERAMIDE IN SEIZURE-INDUCED PROGRAMMED CELL DEATH

## Hippocampal Programmed Cell Death after Status Epilepticus: Evidence for NMDA-receptor and Ceramide-mediated Mechanisms

Mikati MA, Abi-Habib RJ, El Sabban ME, Dbaibo GS, Kurdi RM, Kobeissi M, Farhat F, Asaad W

Epilepsia 2003;44:282–291

PURPOSE: Status epilepticus (SE) can result in acute neuronal injury with subsequent long-term age-dependent behavioral and histologic sequelae. To investigate potential mechanisms that may underlie SE-related neuronal injury, we studied the occurrence of programmed cell death (PCD) in the hippocampus in the kainic acid (KA) model.

METHODS: In adult rats, KA-induced SE resulted in DNA fragmentation documented at 30 hours after KA injection. Ceramide, a known mediator of PCD in multiple neural and nonneural tissues, increased at 2 to 3 hours after KA intraperitoneal injection, and then decreased to control levels before increasing again from 12 to 30 hours after injection. MK-801 pretreatment prevented KA-induced increases in ceramide levels and DNA fragmentation, whether a reduction in seizure severity occurred or not (achieved with 5 mg/kg and 1 mg/kg of MK-801, respectively).

RESULTS: Both ceramide increases and DNA fragmentation were observed after KA-induced SE in adult and in P35 rats. Ceramide did not increase after KA-induced SE in P7 pups, which also did not manifest any DNA fragmentation. Intrahippocampal injection of the active ceramide analogue C2-ceramide produced widespread DNA fragmentation, whereas the inactive ceramide analogue C2-dihydroceramide did not.

CONCLUSIONS: Our data support the hypotheses that (a) *N*-methyl-D-aspartate–receptor activation results in ceramide increases and in DNA fragmentation; (b) ceramide is a mediator of PCD after SE; and (c) age-related differences occur in PCD and in the ceramide response after SE. Differences in the ceramide response could, potentially, be responsible for observed age-related differences in the response to SE.

## COMMENTARY

Status epilepticus leads to the death of neurons in several regions of the brain, particularly the hippocampus. The majority of status epilepticus–induced cell death occurs by necrosis—neurons die via excitotoxicity, mediated by glutamate, free radicals, and calcium. In addition to necrosis, status epilepticus also causes death of neurons through programmed cell death (PCD). In PCD, biochemical cascades activate specific proteolytic enzymes (caspases) that cleave DNA into small, oligonucleosomal fragments (1,2). The process, known as "DNA laddering," is identifiable by the terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling (TUNEL) staining technique. The morphologic correlate of PCD is apoptosis, which has a characteristic histologic appearance of cellular shrinkage, nuclear and cytoplasmic condensation, and chromatin clumping, with relative preservation of cytoplasmic organelles.

The relative contribution of necrotic versus apoptotic cell death after seizures has received considerable attention in the recent literature (3). The distinction between necrotic and PCD is somewhat blurred, as free radical damage can occur in apoptosis, and PCD can result in necrosis (4). Nevertheless, envisioning PCD and necrosis as discrete entities allows consideration of possible sites of intervention in the two types of cell-death processes. The mechanisms of cell death after status epilepticus are of critical concern in epilepsy research. The information might unravel not only important mechanistic questions (e.g., why is the immature brain relatively resistant to status epilepticus–induced cell death?) but also potentially therapeutic ones (e.g., can the various pathways of PCD after status epilepticus be exploited for clinical benefit?)

In PCD, neuron death is the culmination of a variety of mechanisms involving biochemical pathways that are both extrinsic and intrinsic to the cell. The extrinsic pathway of PCD involves activation of a cell-surface "death receptor," whereas the intrinsic pathway entails release of proapoptotic factors from mitochondria. Both of these signals converge with activation of caspases that mediate apoptotic cell death. Ceramide is a sphingolipid, the expression of which is increased markedly by a variety of cellular stresses, including ischemia, oxidative stress, cytokines, ionizing radiation, and chemotherapeutic agents (5). For example, an agonist of the extrinsic pathway, tumor necrosis factor (TNF), leads to an increase in ceramide, with subsequent initiation of PCD and apoptosis via multiple intracellular pathways including cathepsin D, protein kinases, and serine/threonine protein phosphatases (6,7). The effect of seizures on ceramide levels and the role of ceramide in seizure-induced PCD were not investigated before the publication of the study by Mikati et al.

In this report, Mikati et al. demonstrated convincingly that ceramide levels increase as a response to kainic acid (KA)-induced status epilepticus in the adult rat brain. The increase in ceramide is time, region, and age specific. Two peaks of elevated ceramide are seen, at 2 to 3 hours and again at 12 to 24 hours after KA administration. The ceramide increase is predominantly localized to the hippocampus and occurs in rats older than 35 days but not in 7-day-old rats. By using several markers of PCD (e.g., TUNEL stain, Hoechst nuclear stain), the investigators showed that DNA fragmentation (a marker of PCD) correlates with the ceramide increase. The ceramide-associated PCD is *N*-methyl-D-aspartate (NMDA)-receptor dependent because both the seizure-induced increase of ceramide and the occurrence of PCD are blocked by pretreatment with the NMDA-receptor antagonist MK-801. Injection of exogenous ceramide (but not its inactive analogue) induces DNA fragmentation in the hippocampus. Therefore, the authors conclude that ceramide is an important intracellular mediator of PCD after status epilepticus in the adult brain but not in the immature brain.

Is the seizure-induced increase in ceramide an integral part of the PCD pathway or an epiphenomenon? The authors favor the former explanation, and their data are compelling. They have identified a novel pathway by which seizure-induced PCD can occur. It is now necessary to dissect the cell-death cascade further to determine whether intervention at various points can allay the final throes of cell death.

*by Carl E. Stafstrom, M.D., Ph.D.*

## References


1. Friedlander RM. Apoptosis and caspases in neurodegenerative diseases. N Engl J Med 2003;348:1365–1375.
2. Vila M, Przedborski S. Targeting programmed cell death in neurodegenerative diseases. Nat Rev Neurosci 2003;4:2365–2375.
3. Bengzon J, Mohapel P, Ekdahl CT, Lindvall O. Neuronal apoptosis after brief and prolonged seizures. Prog Brain Res 2002;135:111–119.
4. Fujikawa DG. Confusion between neuronal apoptosis and activation of programmed cell death mechanisms in acute necrotic insults. Trends Neurosci 2000;23:410–411.
5. Liou AKF, Clark RS, Henshall DC, Yin X-M, Chen J. To die or not to die for neurons in ischemia, traumatic brain injury and epilepsy: A review on the stress-activated signaling pathways and apoptotic pathways. Prog Neurobiol 2003;69:103–142.
6. Levade T, Malagarie-Cazenave S, Gouaze V, Segui B, Tardy C, Betito S, Andrieu-Abadie N, Cuvillier O. Ceramide in apoptosis: A revisited role. Neurochem Res 2002;27:601–607.
7. Pettus BJ, Chalfant CE, Hannun YA. Ceramide in apoptosis: an overview and current perspectives. Biochim Biophys Acta 2002;1585:114–125.