Experimental Febrile Seizures Require an Undetermined Factor for Induction of Hippocampal Sclerosis in Immature Rat Brain

Serial MRI after Experimental Febrile Seizures: Altered T_2 Signal without Neuronal Death

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Whereas most febrile seizures (FSs) carry a benign outcome, a subpopulation of individuals with prolonged FSs are at risk for later temporal lobe epilepsy. Signal changes on MRI may provide early markers for changes in neuronal integrity that may promote epileptogenesis in such individuals. Here, we used serial MRIs, obtained before and at several times after experimental prolonged FSs, to determine the prevalence and distribution of signal changes on T_2-weighted images and to investigate the pathologic substrates leading to these changes. Seventy-five percent of immature rats with experimental prolonged FSs had abnormal T_2 signal enhancement at 24 hours, and 87.5%, at 8 days after the seizures. The altered T_2 values involved the dorsal hippocampus (75%), the piriform cortex (87.5%), and the amygdala (25%). However, these changes were not accompanied by evidence of neuronal injury or death in these regions, as assessed by using the Fluoro-Jade method. Thus, experimental prolonged FSs lead to relatively frequent abnormal MRI signal in "temporal lobe" structures. Although these changes do not signify cell death, they may denote pathologic cellular processes that promote epileptogenesis.

COMMENTARY

Causality among preexisting hippocampal pathology (e.g., abnormal neuronal migration), prolonged febrile seizure activity, development of hippocampal sclerosis, and epileptogenesis is one of the most debated issues in epilepsy literature, particularly regarding the clinical situation in which prolonged complex febrile seizures in children cause hippocampal sclerosis and epileptogenesis. Here Dube and collaborators show that induction of hyperthermic seizures, lasting for 20 minutes in normal immature rat brain at P10, does not lead to neurodegeneration when assessed up to 8 days later—even though MRI shows changes in T_2-weighted signal intensity in the hippocampus, thalamus, and piriform cortex. This study supports the idea that prolonged seizures associated with elevated body temperature are not enough to trigger hippocampal sclerosis. In addition to seizure activity, something, as yet unknown, is needed to recapitulate the pathology in a subpopulation of patients with prolonged complex febrile seizures.

It is generally acknowledged that the degree of neuronal damage caused by prolonged seizure activity is less severe in the immature rat brain than in the adult rat brain (1,2). One of the factors critical in determining the severity and extent of neurodegeneration is the duration and spread of seizure activity (3,4). In the study of Dube and coworkers, seizure activity lasted for 20 to 22 minutes. Even in studies with adult rodents, 20 to 22 minutes is at the lower end of seizure duration correlated with inducing substantial neuronal death. Thus, one might ask: "What about longer seizures?" Interestingly, however, 20-minute-long experimental febrile seizures trigger long-lasting changes in the expression of subunits of hyperpolarization-activated and cyclic nucleotide-gated (HCN) cation channels, which mediate h-current and, eventually, lead to epileptogenesis in at least 30% of rats (5,6). In other words, experimental febrile seizures without preceding hippocampal pathology are significant enough to trigger long-lasting molecular alterations and epileptogenesis, yet do not produce classic hippocampal sclerosis.

Is it possible that the development of hippocampal sclerosis was missed in this study? Dube and collaborators used T_2-weighted MRI in 4T to assess tissue changes before seizure induction and after 24 hours and 8 days. Two issues in this study relate to timing that may be important regarding the formation of hippocampal sclerosis. First, the investigators selected 24 hours after the induction of febrile seizures to be the initial time point for histology and MRI. Some neuronal populations, such as hilar cells in the dentate gyrus, are highly sensitive to damage, and typically, hilar cell death occurs within a few hours after epileptogenic insults (7,8). Because of phagocytosis, remnants of these cells may not be visible in Fluoro-Jade B staining at 24 hours after insult. Furthermore, small changes in hilus or dentate gyrus might not have been detectable by the type of MRI used, even if the imaging had been performed earlier, because there was limited spatial resolution ($0.51 \times 0.25 \times 1.1$ mm^3). Second, the last time point analyzed was 8 days after febrile seizures. At this time, the hippocampal T_2-weighted

signal alterations were actually more pronounced than at 24 hours, indicating the occurrence of some progressive tissue changes. As both experimental and human studies have shown after more severe seizures, such as status epilepticus (SE), the development of hippocampal atrophy can continue for weeks to months, both in mature and immature animals, and can represent the secondary damage triggered by epileptogenic insult (9,10).

Whether any delayed neurodegeneration will occur in the hippocampus of rats with prolonged febrile seizures remains to be studied. As the histologic analysis previously published by the same group has shown, it is unlikely that any layer-specific damage of the hippocampus would develop in these animals (11). The possible delayed loss of select subpopulations of neurons is, however, of interest, particularly because about 30% of these animals develop spontaneous seizures in a 6-month follow-up. This model provides an exciting opportunity to address the following questions: (a) do recurrent spontaneous seizures that develop after prolonged febrile seizures contribute to the development of hippocampal sclerosis, and (b) consequently, do they lead to hippocampal pathology that is found in a subpopulation of drug-refractory patients with a history of prolonged febrile seizures.

As in chemically or electrically induced SE models, the present study shows substantial interanimal variability in T_2-weighted signal intensity. Theoretically, variability in T_2-weighted signal intensity would provide a range of values to identify those animals that are at risk for poor outcome after prolonged febrile seizures. Unfortunately, the authors present the data as mean values in bar graphs rather than showing the data of individual animals. It was not possible to find a definition for abnormal T_2-weighted signal intensity, even though the authors repeatedly talk about percentages of animals with abnormal T_2-weighted signal intensity. Finally, T_2-weighted signal intensity rather than absolute values of T_2 were measured. The use of T_2-weighted imaging requires normalization of the signal intensity to the adjacent normal-appearing tissue and complicates the interpretation of the results, as measured values inevitably contain small contributions from factors like radiofrequency coil homogeneity, magnetization transfer, and T_1. Some of these factors were carefully taken into account by selecting an appropriately long repetition time and by using external phantoms, yet the use of absolute T_2 would probably have made it possible to distinguish smaller differences and produce physically meaningful values that would be more easily comparable to results from other laboratories.

Two caveats are related to these inaccuracies. The first concerns the difference between the interassay variability in T_2-weighted measurement and the cutoff value for "abnormal T_2 signal." This issue relates to clinical discrepancies; for example, hippocampal abnormalities were found in up to 88% of rats, but as the authors' recent observations show, only 30% of the rats developed epilepsy. This study suggests that T_2-weighted signal-intensity abnormality appears in a substantially larger population of animals than does the subsequent epileptogenesis. Interestingly, a subpopulation of rats shows epileptiform activity in EEG but no seizures by 6 months after febrile seizures (6). The real correlation between the animals with early abnormalities in T_2-weighted signal intensity and epilepsy remains to be investigated. The second caveat involves the issue of using T_2-weighted signal-intensity abnormality as a surrogate marker for later outcome. Relevant questions include the following: is it the degree of severity of abnormality that determines the outcome, and could some other MRI parameters better correlate with the outcome?

A significant question raised by the data presented is what caused the abnormalities in T_2-weighted signal intensity, if it was not neurodegeneration? In general, these types of changes are associated with vasogenic edema formation, which is often, but not always, associated with cell loss. At 24 hours, it is likely that disturbed water homeostasis, as a consequence of seizures, contributed to T_2-weighted signal intensity. This effect can be completely reversed and is not necessarily associated with development of damage in subsequent weeks (10).

An interesting finding is that T_2-weighted signal intensity seems to increase further from 24 hours to 8 days. This finding may be associated with some progressive changes. T_2 relaxation in tissue is induced by extremely complex dynamic interaction of water with surrounding molecules and structures. Although T_2 is potentially sensitive to many changes taking place (e.g., in water homeostasis or exchange processes, cytoskeleton, or myelinization), it is extremely difficult, or possibly misleading, to associate its changes with any specific pathologic process. The finding by Dube and collaborators that an increase in T_2-weighted signal intensity is not necessarily associated with cell death, not only very nicely supports this view but also demonstrates that some progressive MRI changes, indeed, can be detected after induction of febrile seizures.

The question remains whether T_2 weighting, obtained by fast spin-echo sequence, is the optimal contrast to monitor these changes. Likely the most versatile imaging technique, MRI provides a number of different techniques for detection of the seizure-induced alterations. For example, magnetization transfer and diffusion tensor imaging are more likely to detect specific changes in myelinization or fiber pathways. These and several other novel approaches, however, must be carefully and further studied in animal models of epileptogenesis. The work by Dube and collaborators is among the first to demonstrate how MRI detects changes in immature brain after seizures. This kind of work is needed to increase the understanding of progressive changes during epileptogenesis and epilepsy as well as to

increase the understanding of how different MRI parameters are related to pathologic processes.

The work of Dube and collaborators elegantly starts to separate the critical factors associated with epileptogenesis after prolonged seizures coupled with high body temperature. Perhaps the fact that the model used does not recapitulate all features of the phenotype associated with prolonged complex febrile seizures in humans is actually an asset for the model. As the data show, prolonged febrile seizures in normal brain can trigger epileptogenesis without hippocampal sclerosis. It remains to be seen what additional factors are needed in experimental models to produce the full spectrum of long-term clinical phenotype after prolonged complex febrile seizures in humans. Potential factors include genetic background or genetically programmed lesion, preceding focal brain damage, and more prolonged seizure activity. Development and investigation of the prolonged febrile seizure model is a challenge.

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