ANIMAL MODELING OF POSTSTROKE SEIZURES AND EPILEPSY: 5-YEAR UPDATE

Kevin M. Kelly, MD, PhD

Professor of Neurology, Drexel University College of Medicine, Director, Center for Neuroscience Research, Allegheny-Singer Research Institute, Allegheny General Hospital, Pittsburgh, Pennsylvania

Poststroke seizures and epilepsy have been described in numerous clinical and epidemiological studies over many years. In contrast, the pathophysiological events occurring in injured brain that establish poststroke epileptogenesis and epilepsy are not known. However, in the last several years, animal modeling has made significant inroads toward an improved understanding of the progressive biochemical, anatomical, and physiological changes associated with both early and late seizures following stroke. A review of animal studies of poststroke seizures and epilepsy is presented.

Clinical studies and animal models of poststroke seizures and epilepsy were reviewed in this journal 5 years ago (1). At that time, well-designed clinical trials and population studies had addressed several critical clinical issues, generated important findings, and substantially improved the understanding of the occurrence of poststroke seizures and epilepsy (2–6). By comparison, in vivo animal modeling had markedly limited development (7–11). Since that time, several in vivo studies have made significant advances in describing both early and late seizure occurrence following cerebral ischemia and infarction. In a clinical setting, early seizures are considered to be provoked seizures (i.e., occurring within 1–2 weeks following stroke) and caused by the acute metabolic and physiological derangements associated with acute infarction. Late seizures occur after resolution of the acute phase of infarction and are considered to be unprovoked seizures that originate from areas of partially injured brain where neuronal networks have undergone anatomical and physiological alterations, predisposing them to hyperexcitability and synchronization. In the past, two late seizures defined an epileptic condition; however, a recently proposed definition of epilepsy includes an enduring alteration in the brain and at least one seizure (12). These clinical definitions were applied to describe seizure occurrence in a relatively small group of recent animal studies that used either large or small artery occlusion models to generate focal ischemia and infarction. The major findings of these studies are reviewed.

Middle Cerebral Artery Occlusion

Middle cerebral artery occlusion (MCAO) is a standard in vivo large artery occlusion model employed by numerous laboratories to cause focal cerebral ischemia and infarction. MCAO has been used most extensively to study the pathophysiology of stroke, different neuroprotective strategies, and measures of functional outcome following recovery from stroke. MCAO is most commonly performed by insertion of a filament in the artery to cause its occlusion (intraluminal filament model). Another technique, injection of endothelin-1 near the MCA, induces spasm of the artery and resultant ischemia. Similar to MCA-distribution strokes in humans, MCAO can produce a large cortical and subcortical infarct ipsilateral to the occlusion, which is characterized by an infarct core and a surrounding volume of partial cellular injury and death (i.e., the ischemic penumbra). In most rat strains, distal MCAO does not generate a consistent size or distribution of infarct because of the animals' overall vascularity. Importantly, the hippocampus of rats can lie within the region of ischemic injury because it is perfused by the posterior cerebral artery, which can be blocked by MCAO (13). In humans, the hippocampus is typically unaffected by occlusion of the MCA because it is supplied by the anterior choroidal and posterior cerebral arteries.

Early Seizures

In initial studies, which combined MCAO by intraluminal filament with concomitant EEG studies using adult Sprague–Dawley rats, MCAO resulted in large cerebral infarcts and rhythmic spike-wave discharges in cortex ipsilateral to the occlusion, during both ischemic and reperfusion periods (2–5). Building on these results, Hartings et al. performed routine and quantitative EEG analysis of animals up to 72 hours following transient (with reperfusion) or permanent (no reperfusion) MCAO (14). Their important, basic finding was that multiple generalized or ipsilateral electrographic seizures occurred within

Address correspondence to Kevin M. Kelly, Professor of Neurology, Drexel University College of Medicine, Director, Center for Neuroscience Research, Allegheny-Singer Research Institute, 320 E. North Avenue, Allegheny General Hospital, Pittsburgh, Pennsylvania 15212. E-mail: kelly@wpahs.org

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the first 2 hours following MCAO. These ictal discharges were not accompanied by any behavioral manifestations of seizure activity (e.g., convulsions, motor arrest) in the unanesthetized and ambulatory animals. Additionally, periodic lateralized epileptiform discharges occurred primarily ipsilateral to the lesion and persisted throughout the 72-hour monitoring period in most of the animals tested. These results were thought to mirror several electroclinical aspects of the immediate and early phases of human stroke and suggest the potential for subsequent epileptogenicity to develop in the peri-infarct area (15). The effects of antiepileptic drugs on these nonconvulsive seizures were tested in subsequent studies (16,17). These studies found that ethosuximide and gabapentin attenuated the electrographic seizures in a dose-related manner and had the best therapeutic profile of all the antiepileptic drugs tested.

Using the endothelin-1 model of MCAO and video-EEG monitoring of juvenile Sprague–Dawley rats, Karhunen et al. assessed electrobehavioral events in the initial 2-hour period (18). Electrographic seizures without behavioral manifestations were recorded in virtually all of the endothelin-1-injected animals; some saline-injected sham animals also demonstrated early seizures, which resulted in the death of one animal several hours after the injection. These studies corroborated the earlier findings of Hartings et al. and indicated that early seizures were not dependent on the particular technique used to produce MCAO.

Late Seizures

The first study to assess the possibility of late seizure occurrence following transient MCAO was performed by Karhunen et al. using the intraluminal filament model (19). A small cohort of Sprague–Dawley rats was assessed for the development of spontaneous seizures by continuous video-EEG for 1 week at 3, 7, and 12 months postlesioning. No evidence of either electrographic or behavioral seizure activity was found in any animal at any of the time points studied. Following this study, the investigators assessed relatively large cohorts of animals, lesioned by endothelin-1-induced MCAO and evaluated with intermittent 2-week continuous video-EEG monitoring at 6 and 12 months postlesioning (18). Lesion formation by endothelin-1 was variable and only one animal (in the 6-month group) of 26 lesioned animals developed epileptic seizures. Histological analysis of the epileptic animal's brain showed a 10-mm^3 infarct of the ipsilateral basal ganglia. Interictal spiking was detected in 9 of the 26 lesioned animals. Interestingly, early seizures did not predict the development of epilepsy or interictal epileptiform activity in any animal. Taken together, these two pioneering studies provided the first long-term video-EEG monitoring results of rats lesioned by MCAO and suggested that the epileptogenicity of MCAO-induced ischemia and infarction was low.

Middle Cerebral and Common Carotid Artery Occlusion

A combination of transient unilateral MCAO and common carotid artery occlusion (MCAO/CCAO) has been used to create reproducible cortical infarcts, while sparing subcortical structures and hippocampus (20). The MCAO/CCAO technique is performed by placing a small wire underneath the MCA, proximal to its major bifurcation and distal to the lenticulostriate arteries. The artery is lifted by the wire, which is rotated clockwise. The CCA is occluded using atraumatic aneurysm clips. After 3 hours, reperfusion is established by first removing the aneurysm clips from the CCA and then rotating the wire counterclockwise, removing it from beneath the MCA. Kelly et al. used MCAO/CCAO in juvenile Long–Evans rats, monitoring them by intermittent video-EEG recordings for 6 months to determine whether restricted cortical infarction was capable of generating either focal or generalized epileptic seizures (21). The main finding of this study was that large, consistently sized cortical infarcts did not independently generate either focal or generalized epileptic seizures. However, assessment of the video-EEG recordings revealed that sham-operated animals demonstrated interictal focal or restricted bilateral 7 to 8 Hz spike-wave discharges (lasting 1–2 seconds), without behavioral change, and that ictal generalized 7 to 8 Hz spike-wave discharges (absence seizures) were prolonged, frequent, and associated with motor arrest of the animal. Lesioned animals demonstrated interictal spike-wave discharges similar to controls (except that focal discharges were more numerous relative to bilateral discharges) and ictal spike-wave discharges that were of shorter duration and less frequent than controls. Lesioned animals also displayed decreased hemispheric and regional spectral power at ~7 and 15 Hz—directly related to the reduced occurrence of ictal spike-wave discharges—compared with controls. These studies corroborated the results of Karhunen et al. (18,19) and suggest that low or absent generation of poststroke epilepsy in lesioned juvenile animals is relatively independent of the arterial occlusion model or rat strain used.

Photothrombosis

An alternative to large artery occlusion models of cerebral ischemia and infarction is the small artery occlusive technique of cortical photothrombosis and brain infarction, using the photosensitive dye, rose bengal (22). Rose bengal is injected into the animal and activated in brain vasculature by an external light beam that is focused on and penetrates the translucent skull and underlying cerebral cortex. The activated dye generates singlet oxygen, causing peroxidation of endothelial cell membranes and occlusive platelet aggregation. Subsequent thrombus formation, vascular stasis, and vasogenic edema lead to focal cortical infarction and necrosis. This method is well characterized, relatively

noninvasive, and produces cortical infarcts that extend to the subcortical interface, allowing for their selective placement with reproducible area, depth, and location. However, photothrombosis preferentially occludes small-diameter pial cortical vessels, an end-arterial mechanism different from most cases of thrombotic stroke in humans. The thrombus, formed as a result of endothelial damage by singlet oxygen, contains virtually no fibrin. Endothelial discontinuities, such as luminal surface microrupture caused by singlet oxygen peroxidation, lead to early blood–brain barrier opening and severe vasogenic edema. Because the tissue bordering the lesion is destroyed by the rapid development of severe edema, the penumbral area is very small compared with that of arterial occlusion models, extending only ~500 mm from the infarct core (23,24).

Compared with MCAO, photothrombosis results in much smaller infarct volumes (~20 mm^3 vs ~100–300 mm^3). Initial studies using video-EEG monitoring of adult Sprague–Dawley and Fischer 344 rats indicated that photothrombotic cortical infarcts could result in brief, recurrent electrical seizures, recorded from perilesional cortex associated with behavioral arrest of the animal and occurring 1 to 2 months after lesioning (11). These studies gave rise to subsequent investigations that assessed late seizure generation following photothrombosis. Technical constraints of the photothrombosis model, to date, have prevented the assessment of possible early seizures.

Late Seizures

Based on the results of the initial study of photothrombosis applied to late seizure detection (11), Kharlamov et al. developed the photothrombosis model to characterize EEG and behavioral properties of lesioned young adult Sprague–Dawley rats during extended video-EEG recordings obtained by skull screw electrodes (25). Qualitative and quantitative EEG analyses were performed on digital video-EEG records obtained during 6 months of recording. The main finding of this study was that 50% of lesioned animals developed brief (1–3 seconds) focal epileptic seizures ipsilateral to the cortical infarct, characterized by rhythmic spike-wave discharges, with or without behavioral change. Latencies to the first recorded focal spike-wave discharges ranged from 26 to 181 days (mean 107.4 ± 32.2 days). A total of 48 spike-wave discharges were recorded with an average occurrence of 0.215/hour (i.e., 1 seizure every 4.6 hours). Importantly, electrical and behavioral characteristics common to both lesioned and control animals included generalized tonic–clonic seizures (1 naive control; 1 lesioned animal). Additionally, epileptic animals demonstrated increased delta, theta, and low-beta range power ipsilateral to the infarct, which reliably distinguished them from lesioned nonepileptic and control animals.

In a subsequent study, Kharlamov et al. assessed neuropeptide Y protein expression in the brains of both epileptic and nonepileptic animals, after the 6-month monitoring period (26). The main finding of the study was that lesioned epileptic animals with frequent seizure activity demonstrated significant increases of neuropeptide Y protein expression in the cortex, CA1, CA3, hilar interneurons, and in granule cells of the dentate gyrus. Additionally, activated astroglia were detected in the cortex and hippocampus, following lesioning and the development of seizure activity. In general, neuropeptide Y protein expression and morphological changes after cortical photothrombosis were region and pathological state dependent and clearly indicated that significant anatomical changes extended beyond the lesioned cortex in epileptic animals.

In a recent study by Karhunen et al., late seizures were assessed in adult Sprague–Dawley rats following photothrombosis (27). Video-EEG recordings were obtained by skull screw and hippocampal electrodes for 7 to 14 days (24 hour/day) at 2, 4, 6, 8, and 10 months postlesioning. The main finding of the study was that convulsive seizures were recorded in 18% of lesioned animals, with an average seizure frequency of 0.39 seizures/day and a mean seizure duration of 117 seconds. Latencies to the first recorded seizure ranged from 71 to 297 days. Histological analysis of lesioned brains revealed that infarct depths varied from cortical layers I to VI in epileptic animals, which demonstrated light mossy fiber sprouting in the inner molecular layer of the dentate gyrus both ipsilateral and contralateral to the lesion. The findings provide clear evidence of convulsive seizure activity that develops over long periods of time following cortical photothrombosis and is associated with anatomical changes in the hippocampus observed in models of status-epilepticus–induced limbic epilepsy. These results substantially extend the findings from previous studies of the photothrombosis model applied to the study of late seizures (11,25) and provide justification for continued use of the model to explore mechanisms of poststroke epileptogenesis.

In summary, efforts to develop models of poststroke seizures and epilepsy in the last 5 years have yielded important, fundamental findings using a variety of large and small artery occlusion techniques. Results of these studies will allow better-targeted experimental designs for future investigations focused on acquiring a mechanistic understanding of poststroke epileptogenesis and the establishment of the epileptic state.

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