

ADENOSINE PREVENTS KINDLED SEIZURES—AN EFFECT AS SMOOTH AS SILK

Antiepileptic Effects of Silk-Polymer Based Adenosine Release in Kindled Rats. Szybala C, Pritchard EM, Lusardi TA, Li T, Wilz A, Kaplan DL, Boison D. *Exp Neurol* 2009;219(1):126–135. Pharmacotherapy for epilepsy is limited by high incidence of pharmacoresistance and failure to prevent development and progression of epilepsy. Using the rat hippocampal kindling model, we report on the therapeutic potential of novel silk-based polymers engineered to release the anticonvulsant adenosine. Polymers were designed to release 1,000 ng adenosine per day during a time span of 10 days. In the first experiment, rats were kindled by hippocampal electrical stimulation until all animals reacted with stage 5 seizures. Adenosine-releasing or control polymers were then implanted into the infrahippocampal fissure ipsilateral to the site of stimulation. Subsequently, only recipients of adenosine-releasing implants were completely protected from generalized seizures over a period of 10 days corresponding to the duration of sustained adenosine release. To monitor seizure development in the presence of adenosine, adenosine-releasing or control polymers were implanted prior to kindling. After 30 stimulations—delivered from days 4–8 after implantation—control animals had developed convulsive stage 5 seizures, whereas recipients of adenosine-releasing implants were still protected from convulsive seizures. Kindling was resumed after 9 days to allow expiration of adenosine release. During additional 30 stimulations, recipients of adenosine-releasing implants gradually resumed kindling development at seizure stages corresponding to those when kindling was initially suspended, while control rats resumed kindling development at convulsive seizure stages. Blockade of adenosine A₁ receptors did not exacerbate seizures in protected animals. We conclude that silk-based adenosine delivery exerts potent anti-ictogenic effects, but might also have at least partial anti-epileptogenic effects. Thus, silk-based adenosine augmentation holds promise for the treatment of epilepsy.

COMMENTARY

Imagine an epilepsy therapy with the following characteristics. First, there is an anticonvulsant drug that can be delivered focally to the affected hyperexcitable circuit, thereby avoiding the systemic side effects of parenteral drugs. Second, the therapy utilizes an endogenous compound that is already present in a location of the brain where its function is to regulate abnormal excitability. Finally, the treatment not only suppresses seizures but also retards epileptogenesis. As implied by

the findings of Szybala et al. described here, this scenario may soon become reality.

Similar in concept to focal electrical stimulation techniques (e.g., vagus nerve stimulation, brain stimulation), targeted delivery systems can place anticonvulsant medications within discrete epileptic circuits. Novel delivery systems for anticonvulsants are a current research priority (1). Potential methods include implantable drug delivery devices, stem cells designed to release drugs, and gene therapies (2). Each of these methods can limit anticonvulsant action to the local pathological circuit, with a pathology-specific rational drug choice, and avoid systemic side effects. However, each method is an invasive procedure with uncertain long-term effectiveness. Encapsulated

cell lines (fibroblasts, myoblasts) engineered to release adenosine were shown to suppress seizures in kindled rats, though this effect dissipated after 2 weeks (3). Stem cell-derived brain implants, designed to release adenosine by a paracrine mechanism, have been shown to prevent kindling epileptogenesis and kainate-induced spontaneous seizures in rodents; this promising technique is subject to potential immunological complications (4,5). The practical challenges to the development of any of these technologies are daunting. Drugs must be engineered to be released in the right place, in the right amount, and for a sufficient length of time to exert the desired effect of seizure suppression or, optimally, to prevent epileptogenesis.

Szybala and colleagues build upon a growing literature suggesting that the purine, adenosine, a known endogenous anticonvulsant, fulfills many of the roles of a focally delivered epilepsy therapy. Adenosine possesses potent anticonvulsant and neuroprotective properties that depend upon adenosine A₁ receptor activation. When administered systemically, adenosine inhibits kindling and protects against seizures in various other animal models (6). Unfortunately, the parenteral use of adenosine or its analogs is limited by unacceptable side effects, such as sedation and hypotension.

In the present study, the natural anticonvulsant effects of adenosine are exploited by incorporating it into a focally effective, biocompatible form. Previous work has shown proof of principle that adenosine microspheres can be incorporated into porous scaffolds of silk polymers (7,8). When this polymer is implanted into brain tissue, the adenosine is gradually released over time into local tissue at a relatively constant rate. Specifically, Szybala et al. designed a silk polymer scaffold that releases 1,000 ng of adenosine per day for 10 days. The scaffold, measuring 0.6 mm × 3 mm, can be implanted into the infrahippocampal fissure of a rat. The unique profile of adenosine release enabled the authors to design experiments to test both anticonvulsant and antiepileptic actions of adenosine.

Their experiments demonstrate that adenosine release from silk-based polymers suppresses seizures in fully kindled rats and prevents kindling development in rats implanted with the adenosine-containing polymer. First, in fully kindled rats fitted with adenosine-releasing polymers, kindled seizures were completely suppressed, but only during the 10 days in which adenosine release occurred. This experiment provides strong evidence for an anti-ictogenic effect of local adenosine release from the silk polymer.

Next, to evaluate the effect of adenosine release on the process of epileptogenesis, adenosine-releasing polymers were implanted into rats before kindling. Compared with controls that did not receive adenosine, adenosine-releasing polymers delayed onset to all seizure stages, and these animals did not kin-

dle beyond stage 1 seizures, at a time when controls were experiencing stage 5 seizures. Furthermore, if kindling stimulations were stopped during the timeframe that rats were maximally protected by adenosine and then were resumed 9 days later (after the adenosine had run out), the treated group remained fully protected. This result suggests that the treated rats were spared from epileptogenesis, at least partially, by the adenosine released during the initial stimulations and that epileptogenesis was not continuing during the 9 stimulation-free days.

These experiments raise the possibility that focal delivery of an anticonvulsant drug to the brain site where it is most needed, can both stop ongoing seizures and slow epileptogenesis. The prospect of suppressing seizures at the site of their origination, thereby avoiding the adverse effects of systemic administration, would represent a true advance in epilepsy therapeutics. Many technical hurdles remain, including devising ways to control the release rate, developing effective treatment beyond 10 days, and determining a way to release the drug on demand, i.e., when a seizure is imminent. Perhaps combining this technology with other focal methods (e.g., electrical, cell-based) would permit synergism in epilepsy protection.

by Carl E. Stafstrom, MD, PhD

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