

GRAFTS OF ENCAPSULATED FIBROBLASTS ENGINEERED TO RELEASE AN ANTICONVULSANT SUBSTANCE

Grafts of Adenosine-releasing Cells Suppress Seizures in Kindling Epilepsy

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Adenosine is an inhibitor of neuronal activity in the brain. The local release of adenosine from grafted cells was evaluated as an *ex vivo* gene therapy approach to suppress synchronous discharges and epileptic seizures. Fibroblasts were engineered to release adenosine by inactivating the adenosine-metabolizing enzymes adenosine kinase and adenosine deaminase. After encapsulation into semipermeable polymers, the cells were grafted into the brain ventricles of electrically kindled rats, a model of partial epilepsy. Grafted rats provided a nearly complete protection from behavioral seizures and a near-complete suppression of after discharges in electroencephalogram recordings, whereas the full tonic-clonic convulsions in control rats remained unaltered. Thus, the local release of adenosine resulting in adenosine concentrations <25 nM at the site of action is sufficient to suppress seizure activity and, therefore, provides a potential therapeutic principle for the treatment of drug-resistant partial epilepsies.

COMMENTARY

One of the greatest problems facing epilepsy research today is the development of better anticonvulsant therapies. Those that are effective and generate few side effects are unfortunately limited. Some of the most difficult cases are those with complex partial seizures.

A molecular approach, one that is not limited to epilepsy, is the use of cells that are manipulated in culture to release a compound that is anticonvulsant. To date, this has not been effective for epilepsy, but analogous approaches for other diseases are promising. Now a combination of genetic engineering and knowledge of endogenous anticonvulsant mechanisms

has provided a potentially exciting approach, specifically targeted to epileptics with complex partial seizures. Huber et al. have focused on adenosine, a naturally occurring substance that inhibits neuronal activity in a variety of experimental systems. Thus, adenosine acts to decrease glutamate release by a presynaptic mechanism, and also has postsynaptic effects that depress neural activity.

To develop this approach, a cell line was developed that was based on mice that had a deletion in one of the two metabolic enzymes of adenosine (adenosine deaminase; ADA). Heterozygote knockouts (ADA +/-) were crossed with a mouse line that would provide immortalized fibroblasts. Because the ADA knockouts were heterozygotes, the resulting cross led to both immortalized fibroblasts without adenosine deaminase (ADA -/-), and cells that had adenosine deaminase (ADA +/+); the latter provided one of the control cell lines used in the study. To delete adenosine kinase (ADK), thought to be the more important of the two metabolizing enzymes of adenosine, baby hamster kidney cells (BHK) were mutagenized to reduce ADK activity specifically. Cells with a deficiency in ADK were selected by resistance to vidarabine (araA), which produces toxic nucleotides in the presence of ADK. Combinations of cells were encapsulated into a small polymer fiber and implanted into the lateral ventricle.

Prior to implantation, bilateral electrodes were implanted into the hippocampus and were kindled for approximately 15 days, until the stimulus evoked a stage 5 seizure. Stimuli during these 15 days occurred up to 12 times per day, somewhat like the “rapid” kindling approach, and used a stimulus strength just over threshold for an afterdischarge or wet dog shake. The polymer fiber was implanted under pentobarbital anesthesia and then the animals were reexamined with the same stimulus after 4–5 days.

Remarkably, implantation of either the ADA or ADK deficient cells was followed by strong suppression of electrographic activity and behavioral seizures in response to the kindling stimulus, and this remained depressed for weeks. The animals were euthanized after 24 days to verify the placement of the polymer, assess damage at the graft site (there was little evidence based on toluidine blue staining), and examine the polymer for adenosine release and cell death. Remarkably, the

cells could release adenosine for the entirety of the study, although the concentration was always in the low nanomolar range.

One of the aspects of this study that was impressive was the thorough approach and attention to effects that would be drawbacks if this approach were to be used clinically. Thus, potential side effects were investigated by observing whether locomotor, social, or other behavior was altered after the grafts were made. Although methods were not described in detail, no obvious defects were detected by observers who were blind to treatment. Specificity for adenosine receptors was studied in a number of ways. For example, the implantation of ADA-depressed cells was less effective than ADK-depressed cells, and indeed it was found that ADA-deficient cells released less adenosine. To confirm an action on adenosine receptors, the A1 receptor antagonist DPCPX was used. Immediately after an injection of DPCPX, the kindled stimulus was effective in producing electrographic and behavioral seizures. Interestingly, it was again ineffective three days later, and could be restored after a second injection of DPCPX, indicating that continued release of adenosine was both possible and effective in this paradigm.

Another extremely important variable that was examined was the ability of the grafts to produce effects in all rats. To

this end, the investigators made use of the fact that some of the kindled rats develop resistance to the anticonvulsant phenytoin, providing an animal model of epileptics who are pharmacologically intractable. In a small group of rats, the investigators found that the grafts were ineffective in the rats that were resistant to phenytoin. Although the mechanisms underlying drug resistance were not examined, it is important to study this phenomenon in animal models because it is such an important clinical problem. Unfortunately, it seems to be just as much a problem in rodents as man.

One of the limitations of this approach is the fact that, eventually, the release of adenosine declines. Thus, any long-term therapy would require repeated grafts, which is a serious drawback. Yet the authors mention that there currently are "survival-enhancing" genes, such as immortalizing oncogenes, which are constitutively active. This might allow survival of the adenosine-releasing cells for longer periods of time. In addition, bioengineers are currently developing better polymers for this general strategy. Together, molecular and bioengineering advances should further efforts to "trick" biological mechanisms and allow this type of therapy to reach the clinic.

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