433. Gene Therapy of Epilepsy by Adenovirus-Mediated Tetanus Toxin Light Chain Gene Transfer

Article *in* Molecular Therapy · May 2005

DOI: 10.1016/j.ymthe.2005.06.435 · Source: OAI

8 authors, including:

Jun Yang
Cleveland Clinic
17 PUBLICATIONS **203** CITATIONS
SEE PROFILE

Mary Garrity
Case Western Reserve University School of Medicine
26 PUBLICATIONS **304** CITATIONS
SEE PROFILE

Imad Najm
Cleveland Clinic
370 PUBLICATIONS **17,846** CITATIONS
SEE PROFILE

Stephan Chabardes
Grenoble Alpes University Hospital
337 PUBLICATIONS **17,556** CITATIONS
SEE PROFILE

doses of busulfan (20 mg/Kg) on days -3 and -2, and transplanted with 0.8-0.9 x 10^6 unfractionated bone marrow cells transduced with either the vector encoding the antigenic peptide or a control vector containing only EGFP. Three weeks after transplantation the animals were immunized with the MOG40-55 peptide for EAE induction and clinically scored for 30 days. Animals transplanted with marrow cells transduced with the vector encoding the encephalitogenic peptide showed significant protection from EAE (11 of 13 mice did not show any clinical sign of EAE), whereas most animals of the control groups (a total of 30 of 34 mice) developed the disease. Analysis of hematopoietic chimerism and gene transfer rates (EGFP) in the peripheral blood of the animals 21 days after marrow transplantation showed an average level of engraftment of 16.25% (range 0.7 - 66.9%), and a mean transduction rate *in vivo* of 36,1% (range 7.7 - 75.9%). These results indicate that creating molecular chimerism in the murine hematopoietic system using non-myeloablative conditioning is a powerful tool to induce immune tolerance to the transgene product and that this strategy can be applied to tolerize to antigens involved in autoimmune diseases.

433. Gene Therapy of Epilepsy by Adenovirus-Mediated Tetanus Toxin Light Chain Gene Transfer

Jun Yang,[1] Qingshan Teng,[1] Mary E. Garrity-Moses,[1] Thais Federici,[1] Imad Najm,[2] Stephan Chabardes,[2] Michael Moffitt,[3] Nicholas M. Boulis.[1]
[1]Dept of Neurosurgery, Cleveland Clinic Lerner College of Medicine, Cleveland, OH; [2]Dept of Neurology, The Cleveland Clinic Foundation, Cleveland, OH; [3]Dept of Biomedical Engineering, Cleveland Clinic Lerner College of Medicine, Cleveland, OH.

Background: Focused inhibition of excitatory neurotransmission in the area of a seizure focus provides an ideal strategy to treat intractable neocortical epilepsy. Clostridial toxin light chain (LC) inhibits synaptic transmission by digesting vesicle-docking proteins without directly altering neuronal health. We have previously reported synaptic inhibition through adenoviral vector mediated LC expression (AdLC) in neural tissue. In the present experiment, we quantified the impact of cortical LC gene expression on focal seizures elicited in the rat.

Methods: 15 adult male Sprague Dawley rats weighing 280-310 g were divided into the three groups: AdLC, AdGFP, and PBS. Under ketamine anesthesia, a stainless-steel cannula was stereotaxically inserted into the right motor cortex for both local injection of viral vectors or penicillin and EEG recording. Four epidural screw electrodes were implanted in bi-parietal and bi-frontal areas for EEG recording. An additional epidural electrode was placed at the tip of right frontal region to serve as a reference. Six days after surgery, penicillin was injected via the cannula to induce seizure. Continuous EEG monitoring and simultaneous video recording were performed to establish pre-treatment baseline. Four days later, AdLC, AdGFP or PBS was injected via the cannula in each group. Inducement of seizure, EEG and video recording were performed again ten days after viral vector administration. Motor performance was assessed by BBB locomotor score, grip strength and rotarod assays before and after viral vector administration. At the completion of behavioral assessments, brains were extracted for histopathological studies.

Results: Our results demonstrated that AdLC reduced frequency of EEG spike from 56±8 to 25±4 spikes/minute (p=0.04), amplitude from 1099.4±231.9 to 746±240.2 μV (p=0.037), and duration from 1440 to 421.8±105.8 minutes (p<0.001). Clinically AdLC rats showed improvement of seizure in degree and frequency. EEG and clinical manifestation of AdGFP and PBS rats exhibited no significant difference before and after injection. BBB locomotor score, grip strength and rotarod assays showed no statistical difference in all groups before and after vector administration. Histological results revealed that transgene expressed in the area of brain surrounding the cannula. No morphological and cell density difference was detected in all animals tested in the three groups.

Conclusion: Our data show that LC delivered by an adenoviral vector capable of specific neuronal inhibition has therapeutic efficacy for epilepsy in a rat model. Gene expression of LC in the cortex reduces EEG frequency, amplitude, and duration of spike. More importantly, AdLC injected rats showed profound symptomatic improvement. This gene transfer modality bears a promising treatment options for epilepsy therapy. Furthermore, it strongly implicates synaptic transmission in the etiology of seizure spread and maintenance.

434. Long-Term Effects on Retinal Function and Structure Using AAV-Mediated Gene Therapy in Blind RPE65 Null Mutation Dogs

Kristina Narfstrom,[1,6] Greg Tullis,[2] Ragnheidur Bragadottir,[3] Mathias Seeliger,[4] Elizabeth P. Rakoczy,[5] Martin L. Katz.[6]
[1]Veterinary Medicine and Surgery, College of Veterinary Ophthalmology, Columbia, MO; [2]Molecular Biology and Immunology, Medical Sciences, Columbia, MO; [3]Ophthalmology, Ulleval University Hospital, Oslo, Norway; [4]Ophthalmology, University Eye Hospital, Tubingen, Germany; [5]Ophthalmology, Lions Eye Institute, Perth, Australia; [6]Ophthalmology, Mason Eye Institute, Columbia, MO.

We have previously reported on successful gene transfer using recombinant adeno-associated virus-mediated (rAAV) subretinal gene delivery for the dog model of human Leber's Congenital Amaurosis. Congenitally blind dogs were treated at 4-30 months of age with similar doses of the rAAV. RPE65 construct into the right eye.

Retinal function and visual behavior have now been assessed in 5 dogs up to approximately 30 months after treatment and in 2 dogs up to 42 months, the former by using simultaneous, bilateral full-field electroretinography (ERG) at least every 3rd month. All dogs were also evaluated using fundus photography and angiography. Three of the dogs were euthanized at 5, 10 and 30 months, respectively, and their eyes used for morphologic studies.

As early as 4 weeks after treatment, gene therapy resulted in remarkable improvements in visually mediated behavior and in increased ERG responses. Rod ERG amplitudes increased up to 3 months after surgery, but then underwent a gradual decline. There was a peak in ERG cone responses at 9 months after surgery, which were sustained up to 24 months post-operatively. Both rod and cone derived responses had returned to near pre-operative values at 33 months following the gene transfer, however. There was clinical progression of funduscopic lesions even after the successful gene transfer. Lipoid inclusion bodies were abolished from the treatment area, although still prevalent in the more peripheral parts of the treated fundus. Photoreceptor morphology was normalized, both within and outside of the gene transfer treated area.

These results show that AAV-mediated gene therapy is effective in rescuing partial visual function in affected dogs. Rescue is not permanent, however, and warrants modifications in treatment strategy and/or of the construct to achieve more life-long effects.