Surgical Neurology International

OPEN ACCESS

For entire Editorial Board visit : http://www.surgicalneurologyint.com

Editor-in-Chief: **James I. Ausman,** MD, PhD University of California, Los Angeles, CA, USA

Translational Neuroscience

From the bench to the bedside: Everolimus for subependymal giant cell astrocytomas in Tuberous sclerosis complex, optic nerve regeneration, targeted cytotoxins for gliomas

Jason S. Hauptman

Intellectual and Developmental Disabilities Center and Department of Neurosurgery, Geffen School of Medicine at UCLA, Los Angeles, CA

E-mail: *Jason S. Hauptman - jhauptman@mednet.ucla.edu
*Corresponding author

Received: 1 December 10 Accepted: 2 December 10 Published: 14 January 11

Surg Neurol Int 2011, 2:2

This article is available from: http://www.surgicalneurologyint.com/content/1/2/2

This article may be cited as:
Hauptman JS. From the bench to the bedside: Everolimus for subependymal giant cell astrocytomas in tuberous sclerosis complex, optic nerve regeneration, targeted cytotoxins for gliomas Surg Neurol Int 2011;2:2

Available FREE in open access from: http://www.surgicalneurologyint.com/text.asp? 2011/1/2/75587

Key words: Axon regeneration, neuroscience, neurosurgery, tuberous sclerosis complex, targeted cytotoxins

Access this article online

Website: www.surgicalneurologyint.com

DOI: 10.4103/2152-7806.75587

Quick Response Code:

EVEROLIMUS FOR SUBEPENDYMAL GIANT-CELL ASTROCYTOMAS IN TUBEROUS SCLEROSIS[1]

Key Points

- Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterized by multiple hamartomas in various organs, including the brain. TSC is associated with severe and refractory epilepsy, mental retardation, psychiatric disease, and subependymal giant-cell astrocytomas (SGAs).
- TSC is caused by dysregulation of the mammalian target of rapamycin (mTOR), a protein responsible for important cellular processes including growth, proliferation, and protein translation.
- Everolimus, an mTOR inhibitor, has a new role in the treatment of SGA in TSC, with patients receiving Everolimus, experiencing a meaningful reduction in tumor volume, decreased hydrocephalus and intracranial pressure, and a decrease in seizure frequency.
- This is an example of molecular medicine, whereby, the molecular genetics of a disease are exploited for tailored therapeutics.

This month in the New England Journal of Medicine (NEJM), a seminal study was published looking at using an mTOR inhibitor, Everolimus, in children with subependymal giant-cell astrocytomas and tuberous sclerosis. This is an ideal article to review for the 'Bench to Bedside,' because it demonstrates good bench research

that has been successfully translated, to take care of sick children. In a nutshell, tuberous sclerosis complex (TSC) is an autosomal dominant disease characterized by hamartomas, spanning multiple organ systems. Brain hamartomas, which develop as cortical tubers and subependymal nodules, result in seizures in up to 90% of the affected children, as well as mental retardation and neuropsychiatric diseases. TSC results from genetic mutations affecting the genes TSC1 or TSC2, both of which act to suppress another protein called mammalian target of rapamycin (mTOR). When mTOR is overactive, as in TSC, the result is increased cell growth and proliferation. One tumor, which up to 20% of children with TSC get, is the subependymal giant-cell astrocytoma (SGA). Altough standard therapy for SGA has been surgical resection, the advent of mTOR inhibitors has raised a question as to whether drugs such as Everolimus, may reduce the size of these tumors in TSC children. This notion has been supported by many basic science *in vitro* studies of the drug (or a similar mTOR inhibitor), suggesting that it may play a role in the treatment of SGA. In this study, 28 patients with SGA and TSC have received Everolimus for SGA.

The mean time of this therapy was 21.5 months, with all patients except one voluntarily completing a mandatory six-month treatment period and 25 continuing beyond the six months. One patient voluntarily dropped out of the study before the sixth month. Everolimus use was associated with a significant reduction in SGA size. Impressively, during the six-month period, 75% of the patients experienced at least 30% reduction in volume and 32% of the patients experienced at least 50% reduction in tumor volume. Patients on Everolimus also experienced improvements in hydrocephalus and parenchymal dysplasia. In fact, no patients on Everolimus experienced worsening hydrocephalus or increased intracranial pressure, no new SGA lesions developed, and no patients required surgical therapy. Furthermore, patients on Everolimus experienced a clinically significant reduction in the frequency of clinical and subclinical seizures. The quality of life improved in the patients as well, something that one would expect given the other clinical improvements. Given that Everolimus is an immunosuppressant medication, it is not surprising that self-limiting infections and stomatitis were common adverse events. Only four patients had serious adverse effects, with two being related to upper respiratory illness and two related to seizures.

This is an extremely exciting study for several reasons. First, it is the translational result of many years of excellent basic science research. Second, it provides hope for nonsurgical therapy in tuberous sclerosis, a disease that has been traditionally considered a surgical disease (with regards to SGAs and refractory epilepsy from cortical tubers). Third, it opens the door to a further study on Everolimus as an anti-epileptic medication. Finally, it highlights the emerging role of molecular medicine in neurosurgery, a future trend that must be harnessed by the practicing neurosurgeon.

TUNING AROUSAL WITH OPTOGENETIC MODULATION OF LOCUS COERULEUS NEURONS[2]

Key Points

- While it has been traditionally taught that the locus ceruleus is involved in arousal due to its dense noradrenergic projections, evidence for this role has been circumstantial. Due to its location, direct modulation of this region in the past had been difficult.
- Using optogenetic techniques (using engineered ion channels that cause neurons to fire or be silent in the presence of light), the authors show that neurons of the locus ceruleus play a crucial role in the regulation of sleep–wake cycles and locomotion.

Traditional neuroanatomy teaches us that locus ceruleus, a noradrenergic nucleus located within the brainstem, is primarily responsible for an organism's arousal.

This is based (in part) on prior studies showing that neurons regularly (tonically) fire during wakefulness and decrease firing during sleep. Furthermore, these neurons irregularly (phasically) fire in response to stimuli that keep the organism awake. The notion of the locus ceruleus playing a role in arousal also comes from pharmacological studies, where norepiphrine injections cause wakefulness, while antagonists act as sedatives. All of this evidence, however, is indirect. In this study, Carter *et al*. use optogenetics (a way of making certain subsets of neurons either selectively fire or be selectively silent in the presence of particular wavelengths of light) to determine the causal role of the locus ceruleus in arousal.

In order to control the activity of the locus ceruleus neurons, the authors first used a virus containing engineered receptors specifically targeted to locus ceruleus neurons expressing tyrosine hydoxylase (an enzyme necessary for the production of norepinephrine). These engineered ion channels were either halorhodopsin (NpHR), a protein that causes the neurons to cease the firing action potentials when stimulated with light, or channelrhodopsin (ChR2), a protein that causes the neurons to fire the action potentials when stimulated with a different wavelength of light. After confirming that locus ceruleus neurons were expressing the desired channel, the animals were injected with the virus bilaterally. Animals with the NpHR channel were found to have significantly lower levels of norepinephrine following activation with light (as expected, since the locus ceruleus neurons would essentially be 'shut off'). Furthermore,

these animals had decreased wakefulness as demonstrated by Electroencephalography (EEG). Conversely, animals that expressed ChR2 in the locus ceruleus demonstrated abrupt waking from sleep after being exposed to light. As activity of locus ceruleus neurons was increased, the probability of waking from both REM and non-REM sleep increased.

Next, the authors examined the chronic stimulation of the locus ceruleus on animals expressing the ChR2 channel in two different modalities: tonic and phasic. This enabled researchers to recapitulate the normal physiological activity noted in the locus ceruleus in prior studies. They found that prolonged tonic activation of these neurons resulted in increased wakefulness, reduced non-REM sleep, and increased locomotion. These effects were maximal after one hour of stimulation, but declined with five hours of stimulation. Although phasic activation also resulted in increased wakefulness and reduced non-REM sleep, the animals actually displayed decreased locomotion. The effects of phasic stimulation, unlike tonic stimulation, were marked after one hour and five hours of stimulation. This study provides a comprehensive assessment of the function of the locus ceruleus *in vivo*. It is clear that the tonic and phasic activities of these neurons play a crucial role in the modulation of sleep-wakefulness and locomotion. One must also speculate about the role of locus ceruleus in a variety of neuropsychiatric disorders in which arousal is affected. It is not beyond the realm of speculation that the operative neuromodulation of this structure may be a future therapy for disorders in which arousal is dysregulated, such as in narcolepsy.

LONG-DISTANCE AXON REGENERATION IN THE MATURE OPTIC NERVE: CONTRIBUTIONS OF ONCOMODULIN, CAMP, AND PTEN GENE DELETION[3]

Key Points

- Prior studies have shown that various strategies can be employed to enhance axon regeneration after optic nerve injury. These include inducing ocular inflammation, providing cAMP injections into the retina, and deleting the regulatory gene Pten from the retinal ganglion cells (RGCs).
- In this study, the authors show that using a combination of all three of the aforementioned strategies act synergistically to enhance axon regeneration tenfold beyond the controls.
- This model provides critical observations that can potentially be translated to the clinic to help patients with both central and peripheral neural injuries.

Understanding the mechanisms involved in the regeneration of injured axons is critical when employing novel therapeutic approaches to patients with neural injury. In this study by Kurimoto *et al*, the researchers demonstrate key molecular manipulations that enhance the regeneration of damaged retinal ganglion cell (RGC) axons within the optic nerve. It has been previously shown that enhancement of axon regeneration can occur in the setting of several experimental techniques — these include the creation of a controlled inflammatory response within the eye (causing increased expression of genes related to axon growth), deletion of genes that negatively regulate cell growth (an example being the Pten gene), and increasing levels of intracellular signaling molecules, such as, cAMP. Although each of these strategies by themselves only perpetuate a modest regeneration of optic nerve axons after injury, no one has performed all three modalities concurrently. Therein lies the main focus of this study.

First, the authors looked for the optimal concentration of cAMP that would enhance inflammation-induced nerve regeneration. After determining the optimal dosage of the inflammatory agent (Zymosan) to be used in conjunction with cAMP, they demonstrated that the cAMP's ability to enhance inflammation-induced axon regeneration was due to an important inflammatory mediator, termed oncomodulin. This molecule, oncomodulin, was a key player in the ability of the inflammation to promote axon growth (but not necessarily for the inflammation's ability to enhance the survival of RGCs following injury). The authors then went on to show that the reason cAMP and oncomodulin depend on each other for axon regeneration was because cAMP was required for oncomodulin to bind within the retina. When the authors additionally deleted the Pten gene from the RGCs, the ability of the axons to regenerate was impressive: over a tenfold increase in axon regeneration was noted, compared to a twofold increase when inflammation and cAMP were performed without Pten deletion. Equally impressive was the fact that these newly regenerated axons were able to find their appropriate targets in the lateral geniculate with six weeks of injury.

This study is a great example of the important study that is being done in the field of axon regeneration. Although more studies needs to be conducted to further delineate whether these newly recovered axons are able to function appropriately, it is not difficult to imagine that soon neurosurgeons will be able to harness some of these techniques to enhance central and peripheral neural recovery in the clinic.

GENE THERAPY-MEDIATED DELIVERY OF TARGETED CYTOTOXINS FOR GLIOMA THERAPEUTICS[4]

Key Points

- Infiltrative gliomas, such as glioblastoma (GBM),

may be treated by toxins that target specific receptors expressed by tumors, but not normal cells. One such receptor is the IL13 receptor, IL13Rα2. This receptor is expressed by 50 – 80% of tumors cells.

- In this study, the authors devised an adenovirus vector (an inactivated virus used to deliver genetic material into the cells) to create cells capable of expressing a specially-designed exotoxin that targets the IL13Rα2 receptor.
- Expression of the exotoxin resulted in significantly prolonged survival, in a variety of mouse models of GBM.

One of the challenges facing therapy for infiltrative brain tumors is the sparing of normal cells and selective destruction of tumor cells. Prior studies have attempted an innovative approach to targeting GBM cells: Taking advantage of a receptor selectively expressed by 50 – 80% of tumor cells, but not normal brain cells. This receptor, IL13Rα2, was targeted by human IL13 fused to a bacterial exotoxin. Unfortunately, this approach did not move beyond phase III clinical trials, primarily because of the significant neurotoxicity associated with therapy. This neurotoxocity was felt to be due to the binding of the toxin to the natively expressed IL receptors in normal cells. In this study, the authors have attempted to improve on the prior technique by using a mutated form of human IL13 that selectively binds to the IL13Rα2 receptor and not the normal receptors.

The first thing the authors attempted to do was to devise a way of delivering the toxin to the diseased brain tissue and to tightly regulate its levels. They developed an inactivated virus that, after infecting the cells, would give them the ability to express this exotoxin. Furthermore, the cells would only express the exotoxin when the animal was given doxycycline, adding a further regulatory step that would result in a tightly-controlled expression of the exotoxin. After demonstrating that their innovative system would result in the robust expression of the exotoxin in naïve mouse brain, they used it in animals that were transplanted with human GBM cells. This new exotoxin resulted in 40% long-term survival of transplanted mice. This was compared to control mice that had median survival rates of under 50 days. This robust survival effect was replicated in additional GBM models, with one model resulting in 30% long-term survival and another in 50% survival. Clearly, future treatment of GBM will need to focus on molecular targeting and selective destruction of tumor cells. This model provides an interesting insight into the potential techniques that may result in prolonging the survival of patients with GBM.

REFERENCES

1. Carter ME, Yizhar O, Chikahisa S, Nguyen H, Adamantidis A, Nishino S, *et al.* Tuning arousal with optogenetic modulation of locus coeruleus neurons. Nat Neurosci 2010;13:1526-33.
2. Gene therapy-mediated delivery of targeted cytotoxins for glioma therapeutics. Candolfi M, Xiong W, Yagiz K, Liu C, Muhammad AK, Puntel M, *et al.* Proc Natl Acad Sci U S A 2010;107:20021-6.
3. Krueger DA, Care MM, Holland K, Agricola K, Tudor C, Mangeshkar P, *et al.* Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. N Engl J Med 2010;363:1801-11.
4. Long-Distance Axon Regeneration in the Mature Optic Nerve: Contributions of Oncomodulin, cAMP, and Pten Gene Deletion. Kurimoto T, Yin Y, Omura K, Gilbert HY, Kim D, Cen LP, *et al.* J Neurosci 2010;30:15654-63.