Nanoparticle, In Vivo Gene Therapy

Activates Brain Stem Cells

Using customized nanoparticles that they developed, University at Buffalo scientists have for the first time delivered genes into the brains of living mice with an efficiency that is similar to, or better than, viral vectors and with no observable toxic effect, according to a paper published online in the *Proceedings of the National Academy of Sciences* in July 2005.

By Ellen Goldbaum

The paper describes how the UB scientists used gene-nanoparticle complexes to activate adult brain stem cells in vivo, demonstrating that it may be possible to "turn on" these otherwise idle cells as effective replacements for those destroyed by neurodegenerative diseases, such as Parkinson's.

In addition to delivering therapeutic genes to repair malfunctioning brain cells, the nanoparticles also provide promising models for studying the genetic mechanisms of brain disease.

"Until now, no non-viral technique has proven to be as effective as the viral vectors in vivo," says coauthor Paras N. Prasad, PhD, executive director of the UB Institute for Lasers, Photonics and Biophotonics, SUNY Distinguished Professor in UB's Department of Chemistry and principal investigator of the institute's nanomedicine program. "If the clinical trials are successful, this transition from in vitro to in vivo will represent a dramatic leap forward in developing experimental, non-viral techniques to study brain biology and new therapies to address some of the most debilitating human diseases."

Viral vectors for gene therapy always carry with them the potential to revert back to wild-type, and some human trials have even resulted in fatalities.

As a result, new research focuses increasingly on non-viral vectors, which don't carry this risk. Viral vectors can be produced only by specialists under rigidly controlled laboratory conditions. By contrast, the nanoparticles developed by the UB team can be synthesized easily in a matter of days by an experienced chemist.

The UB researchers make their nanoparticles from hybrid, organically modified silica (ORMOSIL), the structure and composition of which allow for the development of an extensive library of tailored nanoparticles to target gene therapies for different tissues and cell types.

A key advantage of the UB team's nanoparticle is its surface functionality, which allows it to be targeted to specific cells, explains Dhruba J. Bharali, PhD, a coauthor on the paper and postdoctoral associate in the UB Department of Chemistry and UB's Institute for Lasers, Photonics and Biophotonics.

While they are easier and faster to produce, non-viral vectors typically suffer from very low expression and efficacy rates, especially in vivo.

"This is the first time that a non-viral vector has demonstrated efficacy in vivo at levels comparable to a viral vector," Bharali says.

In the UB experiments, targeted dopamine neurons—which degenerate in Parkinson's disease, for example—took up and expressed a fluorescent marker gene, demonstrating the ability of nanoparticle technology to deliver effectively genes to specific types of cells in the brain.

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Not So Harmless After All

Ignored bacteria found to be a factor in COPD

A ubiquitous bacterial strain thought not to be involved in chronic obstructive pulmonary disease (COPD) is in fact responsible for two to four million flare-ups of the condition that occur annually in the United States, researchers from the University at Buffalo have shown.

By Lois Baker

The bacterium, Moraxella catarrhalis or M. catarrhalis, often is present in sputum of adults with COPD. Its potential role in the disease, however, was ignored for decades, because studies in the early 1950s had found it to be relatively harmless.

A study published in the July 15, 2005, issue of the *American Journal of Respiratory and Critical Care Medicine* reports that M. catarrhalis was found to be responsible for approximately 10 percent of exacerbations of COPD. Timothy F. Murphy, MD, professor of medicine and microbiology in the UB School of Medicine and Biomedical Sciences, was lead author on the study.

"This paper is the first to study the involvement of M. catarrhalis in a prospective way in adults with COPD," says Murphy. "Using rigorous methods, our work has shown that acquiring M. catarrhalis is strongly associated with the onset of symptoms of an exacerbation.

"People with COPD, estimated to be about 20 million in the U.S., experience one to two exacerbations per year," says Murphy, chief of the Infectious Diseases Division in the School of Medicine and Biomedical Sciences and a pioneer in vaccine development for respiratory disease. "If 10 percent of all exacerbations are caused by M. catarrhalis, that translates to two to four million exacerbations annually."

COPD is the fourth leading cause of death in the U.S. and many of those deaths occur during exacerbations, he notes. "Exacerbations also cause enormous morbidity and health-care costs. They lead to physician visits, emergency room visits, hospital admissions and respiratory failure requiring mechanical ventilation."

In addition to showing that M. catarrhalis is involved in exacerbations of COPD, the researchers also found that patients make immune responses to the bacterium when they acquire it.

"Both of these observations provide lines of evidence that M. catarrhalis is a pathogen for these patients and provide a strong rationale for pursuing the development of vaccines to prevent M. catarrhalis infections in people with COPD," concludes Murphy.

The study involved 104 adults with COPD who were seen at the COPD Research Clinic at the Buffalo Veterans Affairs Medical Center over 81 months.

During this period, patients made 3,009 clinic visits, 560 of which were during exacerbations. Sputum samples were collected at each clinical visit and molecular typing of organisms was conducted, as well as assays to measure immune response.

Researchers identified 120 episodes of M. catarrhalis infections in 50 patients, nearly half of which were associated with flare-ups of COPD. There was no evidence that exacerbations were associated with acquisition of a new strain of another pathogen.

"We know that M. catarrhalis causes ear infections in children," says Murphy. "With these new observations regarding the importance of the bacterium in adults with COPD, we have even more reason to forge ahead with developing a vaccine to prevent M. catarrhalis infections."

Additional researchers on the study were Aimee L. Brauer, research technician in the UB Department of Medicine; Brydon J. B. Grant, MD, UB professor of medicine, physiology and biophysics and social and preventive medicine, and Sanjay Sethi, MD, UB associate professor of medicine.

The study was supported by grants from the Department of Veterans Affairs and the National Institutes of Health. BP

Body Checking Checks Out

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Causes few youth-hockey injuries

Legal checking in hockey—hitting with the shoulder or trunk a player who has the puck or who has just passed the puck—is considered as integral to hockey as tackling is to football. The practice, however, has been much maligned as the cause for injuries among young players, a concern that led the American Academy of Pediatrics to recommend it be prohibited until players are at least 16 years old.

By Lois Baker

But just how harmful is body checking?

This question was recently addressed in a study conducted by UB researchers, the results of which were published in the October 31, 2005 issue of *Medicine and Science in Sports and Exercise*, the official journal of the American College of Sports Medicine.

In the study, which followed 2,630 boys over two seasons, the researchers found that body checks accounted for only 12 percent of injuries while unintentional collisions with the boards, the ice, or between players caused 55 percent of injuries. Seventeen percent of injuries were caused by illegal checking.

Introducing body checking only to teens may actually increase the incidence of more serious injuries, contends the study's lead author Barry Willer, PhD, professor of psychiatry and rehabilitation medicine.

"Bringing body checking into the game at an age when players are big, strong, fast skaters fueled by testosterone could be disastrous from an injury standpoint," says Willer, who played and coached hockey for many years.

Willer's research showed that when body checking was introduced at age nine there was a sudden increase in injuries, most of them minor. But within a year, players had adjusted to giving and receiving body checks and injuries dropped to earlier levels.

Another spike in injuries occurred among the 13 year olds, results showed, which the authors attribute to "increased testosterone levels and concomitant aggressiveness." Injury rates in this group also dropped to near previous levels by the time the boys were age 14.

John Leddy, MD '85, FACSM, a sports medicine physician and coauthor on the study, says the marked increase in injuries among 13 year olds is troubling.

"We think youth hockey leagues may need greater enforcement of the rules among these adolescents as they adjust to changes in their hormone levels," explains Leddy, who is a clinical associate professor of orthopaedics and associate director of the UB Sports Medicine Institute.

Willer suggests that the key to injury prevention is increased skill development, plus a greater emphasis on learning to play "heads-up" hockey. He suggests that body checking may be a key component of teaching this technique to skilled players.

"These younger kids are injured more often by falling into the boards or colliding with each other, in part because they haven't learned to skate or stop well. In addition, it's important to teach a child very early to learn to look toward where he wants to shoot the puck and to 'feel' the puck with his stick, instead of watching the puck. By watching where you are going, you learn to avoid collisions."

Willer points out that checking is not allowed in women's hockey, yet elite women players sustain as many injuries as male players.

The boys in this study were between the ages of 4 and 17 and were enrolled in a Burlington, Ontario, youth hockey program in 2002 through 2004. In addition to the findings on body checking, a primary end point of the study, results showed that injuries were four times more likely to occur in games than in practices.

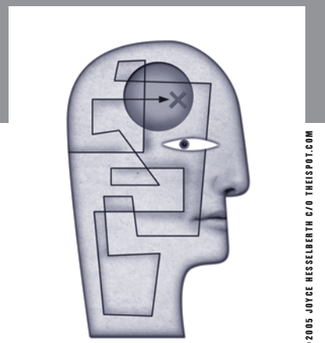
Also, boys who played in the most advanced levels of competition (representative hockey) were six times more likely to be injured than the less skilled house-league players, primarily due to the speed and aggressiveness of play at the top level.

BP

Microtubules under the Microscope

Their role in mental disorders and Parkinson's disease

By Lois Baker



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"Based on these findings, we have identified several ways to stabilize microtubules against the onslaught of rotenone. These results ultimately may lead to novel therapies for Parkinson's disease."

Microtubules are intercellular highways that transport receptors to their working sites in the brain. In recent months, two groups of UB neuroscientists have published studies that identify these structures as possible targets for treatment of mental disorders and Parkinson's disease.

The researchers studying mental disorders are led by Zhen Yan, PhD, associate professor in the Department of Physiology and Biophysics.

"Mental disorders, such as schizophrenia, depression, anxiety and bipolar, are the most prevalent neurological diseases," says Yan. "Studies conducted by the World Health Organization reveal that mental illness ranks second in terms of cause of disability in the United States and that eight of the 10 of the leading causes of disability are mental illnesses."

Yan's group has been focusing on discovering the convergent and unique molecular and cellular mechanisms that underlie the pathophysiology of mental disorders. In the August 19, 2005 issue of the *Journal of Biological Chemistry,* they published a paper describing how destabilization of microtubules interferes with the action of the NMDA receptor (NMDAR), a target of the neurotransmitter glutamate, which plays a critical role in regulating cognition and memory.

"You can think of NMDAR as the cargo moving along a railway consisting of the microtubules cytoskeleton," says Eunice Yuen, a graduate student in Yan's laboratory and lead author of the study.

"Microtubules are hollow cylinders made up of polymers of the protein tubulin," she continues. "Agents that break up, or depolymerize, microtubules disrupt the railway, stop the traffic and reduce the number of cargoes that get delivered to the neuronal surface.

"In turn, fewer NMDA receptors are available on the surface of the neuron to interact with its neurotransmitter, which results in fewer signals being transmitted to critical areas of the brain," explains Yuen. "Defects in neuronal transport are involved in many neurological diseases."

In an earlier paper from Yan's group, which was published in the June 8, 2005 issue of the *Journal of Neuroscience*, the researchers showed that the neuromodulator serotonin, crucial to the treatment of multiple mental disorders, also regulates NMDA receptor function through the mechanism dependent on microtubules.

"We hypothesize that the function of the serotonin receptor known as 5-HT_{1A}R is to suppress the activity of the NMDA receptor by coupling to cellular signaling, which depolymerizes microtubules," says Yuen. "The breakup of microtubules, in turn, interrupts NMDAR delivery to the neuronal surface, resulting in suppression of NMDAR function.

"This evidence shows that serotonin can regulate NMDAR transport along the microtubule cytoskeleton in neurons," she says. "Dysfunction of this regulation may provide a potential mechanism underlying many mental disorders."

The Parkinson's Disease Connection

The researchers studying Parkinson's disease are led by Jian Feng, PhD, assistant professor in the Department of Physiology and Biophysics.

In a paper published in the August 9, 2005 issue of the *Journal of Biological Chemistry,* they describe for the first time how rotenone, an environmental toxin linked specifically to Parkinson's disease, selectively destroys neurons that produce dopamine, the neurotransmitter critical to body movement and muscle control.

They reported that microtubules are a crucial target for intervention because damage to the structures prevents dopamine from reaching the brain's movement center, causing a backup of the neurotransmitter in the transport system. The backed-up dopamine then accumu-

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Microtubules, Cont'd from Page 39

lates in the body of the neuron, where it breaks down and causes a release of toxic free radicals that destroy the neuron.

"This study shows how an environmental toxin affects the survival of dopamine neurons by targeting microtubules that are critical for the survival of dopamine-producing neurons," says Feng, who was senior author on the study.

"Based on these findings, we have identified several ways to stabilize microtubules against the onslaught of rotenone. These results ultimately may lead to novel therapies for Parkinson's disease."

At least 500,000 people in the United States are believed to suffer from Parkinson's disease, which is slightly more common in men than women. The average age of onset is about 60 and, according to the National Institutes of Health, about 50,000 new cases are reported annually, a number that is expected to increase as the population ages.

Feng and colleagues in the Department of Physiology and Biophysics have concentrated their research on the cellular mechanisms of the disease. They are interested specifically in understanding why rotenone destroys neurons that produce dopamine, while sparing neurons that produce other neurotransmitters.

Using cultures of rat neurons, the researchers subjected neurons that produce various types of neurotransmitters to agents that mimic the action of rotenone. The results showed that dopaminergic neurons were destroyed while others survived.

They then combined the treatment with the drug taxol, which stabilizes microtubules and prevents their breakdown. Findings showed that by protecting microtubules, the toxic effect of rotenone on dopamine-producing neurons was greatly reduced.

"Based on these findings, we believe that microtubules are a critical target of environmental toxins, such as rotenone," says Feng, who further explains that the sources for such toxins are not only agricultural chemicals, but the plants themselves.

"While Parkinson's disease has a higher incidence in rural areas and is associated with pesticides and insecticides," he notes, "many microtubule-depolymerizing agents are naturally produced in plants."

By contributing to a better understanding of the action of these toxins, Feng's group is opening up novel avenues for the development of Parkinson's disease therapies by targeting microtubules.

The NMDA studies conducted by Dr. Yan's group were supported by grants from the National Institutes of Health, the National Science Foundation, and an Independent Investigator Award to Yan from the National Alliance for Research on Schizophrenia and Depression. Other contributors to the studies were Zhenglin Gu, postdoctoral associate, Paul Chen, medical and doctoral student in Yan's laboratory, and Qian Jiang, postdoctoral associate in Dr. Feng's laboratory.

The Parkinson's disease study conducted by Dr. Feng's group was funded by a grant from the National Institutes of Health. Additional researchers on the study were Yong Ren, PhD, Wenhau Liu, PhD, Houbo Jiang, PhD, and Qian Jiang, PhD, postdoctoral associates in the Department of Physiology and Biophysics. BP

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Using a new optical fiber in vivo imaging technique (CellviZio developed by Mauna Kea Technologies of Paris), the UB researchers were able to observe the brain cells expressing genes without having to sacrifice the animal.

Then the UB researchers decided to go one step further, to see if they could not only observe, but also manipulate the behavior of brain cells.

Their finding that the nanoparticles successfully altered the development path of neural stem cells is especially intriguing because of scientific concerns that embryonic stem cells may not be able to function correctly since they have bypassed some of the developmental stages cells normally go through.

"What we did here instead was to reactivate adult stem cells located on the floor of brain ventricles, germinal cells that normally produce progeny that then die if they are not used," says Michal K. Stachowiak, PhD, coauthor on the paper and associate professor of pathology and anatomical sciences in the UB School of Medicine and Biomedical Sciences. Stachowiak is in charge of in vivo studies at the UB Institute for Lasers, Photonics and Biophotonics. "It's likely that these stem/progenitor cells will grow into healthy neurons," he says.

"In the future, this technology may make it possible to repair neurological damage caused by disease, trauma or stroke," says Earl J. Bergey, PhD, coauthor and deputy director of biophotonics at the institute.

The group's next step is to conduct similar studies in larger animals.

The UB research was supported by the John R. Oishei Foundation, the National Science Foundation, the American Parkinson Disease Association and UB's New York State Center of Excellence in Bioinformatics and Life Sciences.

Research at UB's Institute for Lasers, Photonics and Biophotonics has been supported by special New York State funding sponsored by State Senator Mary Lou Rath. BP

Ross Eye Institute

advances

Elizabeth Olmsted Ross, MD '39, is a woman with vision and the desire to support it. She dreamed of a unique center that would combine research and eye care and has worked to make it happen. UB's new Ira G. Ross Eye Institute—named in honor of her late husband—broke ground last fall and is scheduled to open in spring 2007.

Olmsted Ross was the motivating force. She donated $3 million to the initiative on the condition that it be matched with another $3 million. Her premise enticed other individuals and foundations to support the center, including well-known local foundations like the John R. Oishei Foundation and the Margaret L. Wendt Foundation, along with nationally prominent organizations such as Research to Prevent Blindness.

> "Having helped raise two-thirds of the $9 million goal, Elizabeth Olmsted Ross now has issued another challenge—a million dollar match that already is drawing responses."

However, Olmsted Ross didn't stop there. Having helped raise two-thirds of the $9 million goal, she now has issued another challenge—a million dollar match that already is drawing responses. Dr. Amar Atwal and his colleagues at Atwal Eye Care have pledged $100,000; the Carlos and Elizabeth Heath Foundation has pledged $100,000; and governmental agencies are expressing interest as well.

The Ira G. Ross Eye Institute will diagnose and treat eye diseases, as well as provide prevention and rehabilitation services. Additionally, it will provide educational programs for both physicians and members of the Western New York community. BP

—Suzanne Chamberlain

Research to Prevent Blindness

Gail Seigel, PhD, receives $50,000 award

Gail Seigel, PhD, assistant professor of ophthalmology and physiology and biophysics in the UB School of Medicine and Biomedical Sciences, has received a $50,000 Sybil B. Harrington Scholar Award from Research to Prevent Blindness to support her research into diseases of the eye.

The award is part of the organization's special scholar program designed to support outstanding young scientists who are conducting research of unusual significance. Research to Prevent Blindness (RPB) is the leading nongovernment supporter of eye research, and has provided hundreds of millions of dollars in grants to advance treatment and cure eye diseases.

Seigel's research centers on chemo-resistant cells in retinoblastoma, an eye cancer caused by the loss of a pair of tumor-suppressor genes, cell death in diabetic retinopathy, high-resolution imaging in retinal disease and corneal repair.

Seigel works under the auspices of UB's Ira G. Ross Eye Institute, a collaborative center for eye and vision care involving the UB Department of Ophthalmology, the research arm (see article, above); the Elizabeth Pierce Olmsted, M.D., Center for the Visually Impaired, the rehabilitation and training arm; and University Ophthalmology Services, the clinical diagnosis and treatment arm. BP

—Lois Baker