A Site to Control

By Lois Baker

$1.6 million grant funds study of neurodegenerative conditions

Only one drug is approved to treat persons with mild to moderate Alzheimer's symptoms, despite the fact that the brain protein at the core of this disease, the NMDA receptor, is known to play a central role in several acute and chronic neurodegenerative conditions that impair learning and memory.

Gabriela Popescu, PhD, assistant professor of biochemistry in the School of Medicine and Biomedical Sciences, is hoping to help change that situation.

Funded by a $1.6 million grant from the National Institute of Neurological Disorders and Stroke, National Institutes of Health (NIH), she is beginning a new study to investigate the mechanisms that control these receptors and to learn how to disrupt certain damaging NMDA receptor functions, while leaving its other important functions intact.

"Understanding how NMDA receptors work will help neuroscientists rationally address neurodegenerative diseases, such as Alzheimer's, Parkinson's and Huntington's, and may provide novel therapies for stroke and schizophrenia," says Popescu, a member of the Neurodegenerative Group in the New York State Center of Excellence in Bioinformatics and Life Sciences at UB.

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>
> —Gabriela Popescu, PhD

"The shortage of useful therapies is particularly frustrating, given the many distinct regulatory sites available on the NMDA receptor. We are very hopeful we can address this urgent need for a better understanding of how NMDA receptor activities are, or can be, controlled."

The NMDA receptor and its neurotransmitter glutamate have been the focus of intense neuroscience research in recent years.

"Ninety percent of all excitatory neuronal signaling in the brain is controlled by glutamate, and half of it requires NMDA receptors," Popescu explains. "We need these proteins for the correct wiring of our brains and throughout life to form and retain memories, to learn new skills and behaviors. We cannot function properly without them.

"Underactivity of NMDA receptors may be a cause of schizophrenia, while overly active NMDA receptors kill neurons, causing devastating brain damage following a stroke," she says. "NMDA receptors also are involved with pernicious illnesses such as Alzheimer's, Parkinson's and Huntington's in ways that are not yet clear."

Gabriela Popescu, PhD

Popescu was lead researcher on a study, published in *Nature* in 2004 that described for the first time how these receptors can act as "frequency discriminators," with the potential capacity to determine whether a neuron will learn to become more or less receptive to future experiences.

Her follow-up article in *Molecular Pharmacology* showed it should be possible to find drugs that regulate certain NMDA receptor functions, while leaving others alone.

"In addition to proving this important principle," says Popescu, "we anticipate that the NIH project also will suggest novel strategies, perhaps combinatorial, to control specifically the receptor properties responsible for pathologic states, while preserving its required functions in synaptic transmission and plasticity.

"A better understanding of how these proteins work holds great hope for addressing neurodegenerative diseases in the future." BP

Infiltration Systems

Pharmaceutical metabolites found in wastewater

When we think of drugs and their side effects, we generally think about what impact they are having on carefully defined patient populations. A recent study by University at Buffalo chemists, however, raises concerns about how pharmaceutical compounds may be impacting targets for which they were never intended.

ILLUSTRATION BY JOSÉ ORTEGA

By Ellen Goldbaum

This is because UB chemists have for the first time identified the metabolites of two antibiotics and a medical imaging agent at wastewater treatment plants.

While this discovery will allow wastewater treatment plants to begin monitoring for these byproducts, it also reinforces concerns about excreted pharmaceutical compounds from wastewater systems that may end up in the water supply, potentially resulting in adverse effects for humans and the environment.

For example, antibiotics and their metabolites can significantly increase antibiotic resistance in the population. Synthetic hormones can act as endocrine disruptors, by mimicking or blocking hormones and disrupting the body's normal functions.

The scientists conducting this research presented their data as part of a day-long symposium on "Degradation and Treatment of Pharmaceuticals in the Environment" that was held in Orlando, Florida. The symposium was chaired by Diana Aga, PhD, assistant professor of chemistry in UB's College of Arts and Sciences and leader of the UB team, whose research was funded by the National Science Foundation.

According to Aga, it has been only in the past five years that analytical chemistry techniques have become sufficiently affordable and practical to allow researchers to detect pharmaceuticals and their metabolites efficiently at the parts-per-billion and parts-per-trillion range.

"Current wastewater treatment processes are optimized to reduce nitrates and phosphates and dissolved organic carbon, the major pollutants of concern in domestic wastes," she says. "However, treatment facilities don't monitor or measure organic microcontaminants like residues of pharmaceuticals and active ingredients of personal care products."

Aga notes that most previous studies looked for drugs' active ingredients in treated wastewater.

"But now we are doing laboratory studies to characterize what these ingredients degrade into during wastewater processing," she adds. "The lesson is that not detecting active ingredients in the effluent doesn't mean the water is clean. The pharmaceuticals we monitored are not degraded completely in the treatment plants; most of them are just transformed into other compounds that still may have adverse ecotoxicological effects."

The researchers have identified the metabolites for sulfamethoxazole and trimethoprim, commonly prescribed antibiotics, and for a synthetic estrogen, a common ingredient in birth control pills and in hormone replacement therapy.

In research published in January in *Analytical Chemistry,* the group also found that iopromide, a pharmaceutical imaging agent that patients consume before taking MRI tests, is barely degraded in the conventional activated sludge process.

However, they found that when conditions in biological treatment systems are optimized for nitrogen removal, this imaging agent does degrade.

Aga explains that these findings have important implications because it means that wastewater treatment processes can be optimized to remove persistent pharmaceuticals in wastewater.

The researchers obtained samples during fall and spring from local wastewater treatment plants in the Western New York towns of Amherst, East Aurora, Lackawanna, Tonawanda and Holland, representing suburban, urban and rural areas. They sampled effluent before and after each water-treatment stage to examine relative efficiencies of each treatment process.

Aga notes that based on the team's findings, a combination of biological, chemical and physical processing techniques probably will be the most successful to remove completely pharmaceutical compounds and their metabolites from wastewater.

"Originally, it was hoped that during the disinfection process, through chlorination or ultraviolet techniques, removal of the drugs that we studied would be enhanced, but, in fact, neither of these is effective," she says.

The researchers did find, however, that most wastewater treatment processes are effective in significantly degrading some common antibiotics, such as ciprofloxacin and tetracycline. BP

Drawn and Quartered

New drug-delivery system uses magnetic fields to home in on targets

By Ellen Goldbaum

Researchers at UB have demonstrated a promising new drug-delivery method in which nanoparticles that contain drugs are magnetically guided to tumor cells.

The new approach, described in *Molecular Pharmaceutics* (online, April 19, 2006), may lead to treatments that exploit the advantages of photodynamic therapy (PDT) and that have the potential to reduce drug accumulation in normal tissues.

The in vitro results showed that magnetically guided delivery to tumor cells of these customized nanocarriers allowed for more precise targeting, while boosting cellular uptake of the PDT drugs contained inside them.

"This is a novel way to enhance drug delivery to cells," says Paras Prasad, PhD, executive director of UB's Institute for Lasers, Photonics and Biophotonics, SUNY Distinguished Professor in the Department of Chemistry, College of Arts and Sciences, and coauthor on the paper.

"The externally applied magnetic field acted as a kind of 'remote control,' directing the nanocarriers to the targeted area in the cell culture," he says.

Once the magnetic field was applied, the concentration of drug inside the tumor cells in the target area increased.

"We have shown that we can use magnetophoretic control to deliver PDT drugs to tumor cells, resulting in increased accumulation inside those cells," explains Tymish Ohulchanskyy, PhD, postdoctoral research scholar with the institute.

The research was conducted with partial funding from UB's New York State Center of Excellence in Bioinformatics and Life Sciences, which is a major supporter of the nanomedicine program at the Institute for Lasers, Photonics and Biophotonics. Prasad is affiliated with the Bioengineering/Tissue Engineering Team at the Center of Excellence.

"The nanomedicine work by Dr. Prasad and his team has far-reaching implications for a variety of disease areas, including neurological disease and cardiac disease," says Bruce A. Holm, PhD, senior vice provost and executive director of the Center of Excellence.

According to Prasad, photodynamic therapy is one of the most promising treatments for cancer; it's also being investigated as a treatment method for cardiovascular, dermatological and ophthalmic diseases.

PDT exploits the propensity of tumors to retain higher concentrations of photosensitive drugs than normal tissues. When exposed to laser light, these drugs generate toxic molecules that destroy the cancer cells.

The main side effect associated with photodynamic cancer therapy is the patient's strong sensitivity to light for four to six weeks after treatment, a result of PDT drugs that accumulate in the skin.

"The magnetically guided drug delivery would allow for the use of lower concentrations of the drug to deliver a therapeutic dose, thus significantly reducing the amount of PDT drug that accumulates in normal tissue," says Prasad.

The UB team achieved these results with a novel nanocarrier system, developed from polymer micelles, which are nanosized, water-dispersible clusters of polymeric molecules.

Prasad explains that polymeric micelles are excellent nanocarriers

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ILLUSTRATION BY ROD COLVIN

"While the team has demonstrated this concept with PDT drugs, Prasad says the technique would be useful in delivering gene therapy, chemotherapy or practically any kind of pharmaceutical treatment into cells."

Recently, scientists completed the first analysis of the genes of a community of human microbes, an accomplishment that has far-reaching implications for clinical diagnosis and treatment of many human diseases.

Results of the analysis, conducted by a team headed by UB microbiologist Steven R. Gill, PhD, appear in the June 2, 2006, edition of the journal *Science*.

"Our Microbial Partners"

Group led by UB scientist publishes first human microbiome analysis

By Lois Baker

ILLUSTRATION BY JAMES STEINBERG

Gill, who conducted the research while at The Institute for Genomic Research (TIGR) with colleagues from TIGR, Stanford University and Washington University, analyzed the DNA of microbes in the human distal gut as a "community-of-the-whole"—the next frontier in the field of genetic research called metagenomics.

"The human genome is an amalgam of human genes and the genes of our microbial 'selves,'" says Gill, associate professor of oral biology in the UB School of Dental Medicine and a member of the Anti-Infectives and Bioconformatics Team in UB's New York State Center of Excellence in Bioinformatics and Life Sciences. "Without understanding the interactions between our human and microbial genomes, it is impossible to obtain a complete picture of our biology," he says.

The human genome lacks some essential enzymes that break down the food we eat into energy essential for survival, a situation that prompts Gill to note that while bacteria could survive perfectly well without their human hosts, humans would be doomed without their bacterial partners.

"The ultimate goal of the work," he says, "is to develop tools for clinicians to use in treating disease. With this kind of knowledge, we can use biomarkers to identify the bacterial population of the individual. Clinicians then can adjust the population of bacteria to make that person well. Such an analysis also would determine which bacteria are resistant to which antibiotics and help determine the proper drug to administer.

In the future, healthy individuals could undergo a metagenomic analysis of their gut to determine their immune status and susceptibility to certain diseases, explains Gill.

Jeffrey I. Gordon, MD, a major contributor to the research from the Center for Genome Sciences at Washington University, notes that this gut "microbiome" project is an important starting point for developing new drugs for 21st-century medicine.

"Our microbial partners have undoubtedly developed the capacity to synthesize novel chemical compounds that help establish and sustain their mutually beneficial relationships with us," says Gordon.

"Prospecting for these 'natural products' and characterizing the pathways through which they operate should provide new insights into the function of many of our human genes, new ways for defining our health, new ways for identifying impending or fully manifest diseases, plus new treatment strategies."

Although scientists have published metagenomic analyses of samples from other environments, including soil and the Sargasso Sea, this is the first publication of an analysis of human-residing organisms. The researchers chose to investigate the colonic microbiome because fecal samples are readily accessible, because the human gastrointestinal tract is the most densely populated microbial community in the body, and because these microbes perform many critical functions.

Samples for the analysis were derived from two unidentified individuals. The researchers know only that one is male and one is female; one is a vegetarian, one is not. Both contributors had received no antibiotics during the past year, ensuring that their population of intestinal flora was "normal" and stable.

Continued on Page 33

"The ultimate goal of the work is to develop tools for clinicians to use in treating disease. With this kind of knowledge, we can use biomarkers to identify the bacterial population of the individual. Clinicians then can adjust the population of bacteria to make that person well."

Device Targets Hospital Infections

BioBlower sterilization technology developed at UB

By Ellen Goldbaum

The device could be made compact enough to turn an ordinary hospital room into an instant isolation unit, Garvey says, or it could be made as large as necessary to install in a building's HVAC unit to provide purified air throughout an entire facility.

An air-sterilization device that was initially developed for the U.S. Department of Defense to protect troops on the battlefield may soon be protecting hospital patients from infections, due to funding from the New York State Office of Science, Technology and Academic Research (NYSTAR).

The funding comes as hospital-acquired infections, many of which are becoming increasingly difficult to treat, are on the rise.

The $674,900 grant from NYSTAR's Technology Transfer Investment Program will allow Buffalo BioBlower Technologies, the UB spin-off company that licensed the technology from UB, to develop a health-care prototype and take it into clinical trials.

"Receiving this NYSTAR grant is a major boost that will help ensure the success of Buffalo BioBlower," says Robert Genco, DDS, PhD, vice provost and director of the UB Office of Science, Technology Transfer and Economic Outreach (STOR). "We are very confident that in the next few years, companies like Buffalo BioBlower and others spun out of the university will make major contributions to economic development in Western New York."

The NYSTAR funding will support construction of a test room for evaluating a prototype for health-care applications.

The goal of the award is the development of a health-care division for Buffalo BioBlower Technologies, potentially creating up to 100 new jobs.

In tests funded by the Department of Defense and conducted in fall 2005, the UB team has shown it can eradicate greater than 99.9999 percent of the spores of an anthrax surrogate in an airstream, according to the researchers.

"That's better than any conventional technology on the market." says James F. Garvey, PhD, UB professor of chemistry in the College of Arts and Sciences and cofounder and chief technical officer of Buffalo BioBlower Technologies with John Lordi, PhD, chief executive officer. Lordi is a research professor in the Department of Mechanical and Aerospace Engineering in the UB School of Engineering and Applied Sciences.

"We input one million live, active spores of a thermally resistant bacterium into the BioBlower and only one live spore came out," says Garvey.

Through compressive heating and pressure oscillations that break up and kill pathogens, the dual-use technology called BioBlower can be expected to rapidly and continuously eradicate even the smallest of airborne biological pathogens, such as bacteria, spores, viruses, influenza including bird flu, pollen and mold.

That contrasts with the current conventional technology, HEPA (High-Efficiency Particulate Air) paper filters, which trap large airborne spores and need to be changed frequently, stored carefully and subsequently destroyed.

"With HEPA filters, the spores are still alive, once they're collected, waiting to infect somebody," Garvey explains. "We kill them at the source."

The issue could not be more critical to the health-care market.

Due to the high incidence of hospital-acquired infections, "the Centers for Disease Control says going to the hospital is the fourth biggest killer in this country," notes Garvey.

The device could be made compact enough to turn an ordinary hospital room into an instant isolation unit, Garvey says, or it could be made as large as necessary to install in a building's HVAC unit to provide purified air throughout an entire facility.

Buffalo BioBlower's administrative office is in UB's New York State Center of Excellence in Bioinformatics and Life Sciences.

Lordi says that's a clear advantage, especially in light of the recent NYSTAR funding.

"We will benefit from interactions with the researchers in the Center of Excellence, as well as with all of the people in health care on the Buffalo Niagara Medical Campus as we learn more about the whole problem of infection control."

In Department of Defense funding announced in July by U.S. Representative Louise Slaughter, Buffalo BioBlower received $1.5 million to develop a prototype for military applications, now being built in space the company rents at Calspan Corp. in Cheektowaga, New York.

The system is one of a handful from around the U.S. that the Department of Defense has chosen to test this fall as a candidate for future procurement.

Other investments in the company and its technology include $200,000 in original Department of Defense funding, matched by $50,000 from UB's Center for Advanced Technology in Biomedical and Bioengineering, and $20,000 from UB STOR's Product Development Fund for studies on the technology's mechanism. BP

Tension Headache, or Not?

Study shows pain may be due to TMJD

By Lois Baker

People whose recurrent headaches have been diagnosed as tension related actually may be suffering from temporomandibular muscle and joint disorder (TMJD).

This is the conclusion of a study headed by Richard Ohrbach, DDS, PhD, UB associate professor of oral diagnostic sciences, in which examiners could replicate tension-headache symptoms in 82 percent of subjects by performing the clinical examination of the temporalis muscle, which is involved in TMJD.

ILLUSTRATION BY KATHERINE MAHONEY

The temporalis muscle is responsible for closing the jaw and is involved in chewing, but these core functions of that pair of muscles often are ignored when the presenting complaint is "headache," as opposed to jaw pain, explains Ohrbach.

"Because headache is so incredibly common, it often is regarded as inevitable, and if sufferers label the pain as 'headache,' they may not seek help," he says. "Or if they do seek help, the label of 'headache' typically will propel the individual to a physician or neurologist for consultation.

"Knowledge about the intersection between jaw pain and headache is not well established, and consequently, jaw pain may be ignored in the differential diagnosis," notes Ohrbach. "This can be most unfortunate for the individual, because TMJD can be very treatable, but if a jaw disorder is ignored, then treatment for the headache may not address all of the factors contributing to the headache."

The current study being led by Ohrbach is part of an $8 million project funded by the National Institute of Dental and Craniofacial Research, the goal of which is to establish valid and reliable TMJD diagnostic criteria. Results will advance the field of TMJD research and aid clinicians in their practices.

Researchers at the University of Minnesota and the University of Washington, in addition to UB, are involved in the project.

An estimated 5–10 percent of the U.S. population suffer from TMJD severe enough to warrant treatment. These patients experience debilitating pain that can destroy quality of life. Diagnosing the disorder is problematic, however, due to overlap with other conditions, says Ohrbach.

TMJD usually involves more than a single symptom, rarely has a single cause and frequently involves multiple factors, including behavioral and emotional responses. Lacking a firm set of diagnostic tools, physicians and dentists often depend on their individual judgment to decide if a patient does or does not have the disorder, he notes.

The diagnostic criteria for TMJD being tested in this project are part of the established Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD). Headache diagnosis is based on the International Headache Society (IHS) guidelines. All examiners were been trained to use the "gold-standard" criteria for tension-type headache established by the IHS.

The study compared the diagnostic procedures for pain and the reproduction of "pain" vs. "headache" during the clinical examination. Procedures included a range of functional and orthopedic tests and standard pain sensitivity to pressure applied to the muscles associated with headache. The types of headaches considered included sub-clinical headaches, tension-type headaches and headaches exhibiting more symptoms than are accepted for tension-type headaches, such as the "mixed headache," migraine or "migraine-type" headaches.

The study involved 583 participants—82.3 percent female and 17.7 percent male—who were recruited as cases from the community based on the presence of symptoms clearly associated with TMJD. Based on IHS criteria, 31.5 percent of participants were diagnosed with tension-type headache by the examiners. BP

Other researchers on the study included Yoly Gondalez, DDS, from UB; John O. Look, DDS, PhD, Eric L. Schiffman, DDS, and Wei Pan, PhD, from the University of Minnesota, and Edmond L. Truelove, DDS, from the University of Washington.

Drawn & Quartered, Cont'd from p. 28

for PDT drugs, which are mostly water-insoluble.

Along with the photodynamic drug, the UB researchers encapsulated inside the nanocarriers iron oxide nanoparticles, which allowed them to respond to externally applied magnetic fields.

In the experiments, nanocarriers were shown to be efficiently taken up by cultured tumor cells in the area exposed to the magnetic field, as demonstrated by confocal microscopy.

While the team has demonstrated this concept with PDT drugs, Prasad says the technique would be useful in delivering gene therapy, chemotherapy or practically any kind of pharmaceutical treatment into cells.

"Because the nanocarriers proved to be significantly stable and because they retained the PDT drugs, we are optimistic that they will be able to deliver a wide range of therapies to tumors or other disease sites in the body without any significant loss in the circulatory system or in normal tissues," explains Prasad.

The team has begun in vivo studies on the new drug-delivery method. Preliminary studies in live animals have indicated that an applied magnetic field can effect a localized accumulation in the tumor site, according to Earl J. Bergey, PhD, deputy director of biophotonics at the institute and a coauthor on the paper. BP

Other coauthors are Ludmila O. Cinteza, PhD, former postdoctoral researcher at the institute; Ravindra K. Pandey, PhD, professor of biophysical sciences at Roswell Park Cancer Institute and research professor at the institute; and Yudhisthira Sahoo, PhD, research assistant professor in the Department of Chemistry. The UB research was funded by the John R. Oishei Foundation, UB's New York State Center of Excellence in Bioinformatics and Life Sciences, and by the UB Interdisciplinary Research and Creative Activities Fund.

For more information on Photodynamic Therapy, see "An Idea That's Taken on a Light of Its Own" in the fall 2004 issue of Buffalo Physician *at www.smbs.buffalo.edu/bp/*

Microbial Partners, Cont'd from p. 29

Metagenomic analysis of the two microbial communities for their potential to carry out necessary functions of human metabolism showed that both had ample concentrations of essential bacteria, but comparison of the two identified significant differences.

One subject was "enriched"—host to more bacteria of a given category than expected—for energy production and conversion, carbohydrate transport and metabolism, amino acid transport and metabolism and several other functions.

"This metagenomics analysis begins to define the gene content and encoded functional attributes of the gut microbiome in healthy humans," states Gill. "In the future we hope to assess the effects of age, diet and diseases such as IBS, cancer and obesity in the microbial community of the distal gut in people living in different environments."

Sampling the gut microbiome periodically, as well as those of other sites, such as the mouth and skin, may allow scientists to determine the effects of environmental change on our "microevolution," says Gill. BP

Additional authors on the paper are Robert T. DeBoy, Claire M. Fraser-Liggett and Karen E. Nelson from TIGR; Mihai Pop from University of Maryland; Paul B. Eckburg and David A. Relman from Stanford University; and Peter Turnbaugh and Buck S. Samuel from Washington University.

The study was funded by grants from the Defense Advanced Research Projects Agency, the Office of Naval Research, the W. M. Keck Foundation, the Ellison Medical Foundation and the National Institutes of Health.