Inhibiting autoimmunity

Scientists at Active Biotech, a Sweden-based drug discovery company, recently reported on their efforts to optimize roquinimex, a previously failed therapy candidate for multiple sclerosis (MS).

Pharmacia & Upjohn, now part of Pfizer, halted Phase III MS studies of Linomide (roquinimex) in 1997 because of unacceptable toxicity. However, the compound’s ability to inhibit experimental autoimmune encephalomyelitis (EAE) in mice and rats—a model system for human MS—and its early promise in human studies make it an advantageous starting point for MS drug design.

To improve the compound for future therapeutic use, Stig Jönsson and colleagues from Active Biotech generated numerous chemical derivatives of roquinimex and determined structure–activity relationships (SARs; *J. Med. Chem.* 2004, 47, 2075–2088). They screened the new compounds for activity against EAE and tested the most active ones for proinflammatory responses in beagles.

The researchers used four substituent classes—alkyls, halogens, acceptors, and donors—to generate derivatives of roquinimex at several positions. First, they made modifications at the 5-, 6-, 7-, and 8-positions of the quinoline ring (R1), finding that EAE potency was enhanced by substitutions at the 5- and 6-positions but not the 7- and 8-positions, with the 5-position changes showing the greatest effects. At the N-carboxamide position (R4), the researchers determined that only the endogenous methyl substitution was effective.

In substituting the methyl group of the N-carboxamide position (R4) for longer alkyl chains, they noted that the EAE activity dropped in all cases, although only modestly for the change from methyl to ethyl. Similarly, the researchers found that substitutions at the para and meta positions of the 3-carboxamide phenyl ring (R3) had little effect on EAE activity, whereas changes at the ortho position had an adverse influence.

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The researchers then examined the ability of some compounds with high EAE activity to trigger a proinflammatory response in dogs. They found that derivatives substituted at the R1 6-position exhibited a proinflammatory response. Likewise, they determined that compounds substituted at the R3 5-position showed a proinflammatory response when R3 was a methyl group but not when it was substituted for an ethyl group.

This led to the discovery of laquinimod, a candidate with improved potency and a better toxicological profile than roquinimex, according to the researchers. The compound is currently in Phase II studies for the treatment of MS.

—RANDALL C. WILLIS
On the critical path

Innovation or stagnation? This is the challenge the FDA posed to itself and to all participants in the drug development process in a white paper released in March.

The report, in essence, examines the much-maligned “pipeline problem”—that is, the recent slowdown in innovative medical therapies reaching patients. The number of applications submitted for new molecular entities and novel biologics has steadily decreased over the last 5–6 years, according to the agency, whereas pharmaceutical R&D spending has almost doubled in that time.

The fundamental issue identified by the FDA—what it calls a “growing crisis”—is that product development is not keeping pace with basic science innovation.

“With so much promising technology in development in the clinical labs, ranging from engineered tissues to new kinds of biologicals and genomics-based treatments, we need to turn the process of bringing these technologies to patients from a costly and time-consuming art form to a well-understood science,” says Mark McClellan, former FDA commissioner and current Centers for Medicare & Medicaid Services administrator.

There is an urgent need, the report stresses, to concentrate on “critical path research” directed at improving the product development process.

This, the agency believes, will primarily require new evaluation tools—such as computer-based models, genomic and proteomic biomarkers, and novel clinical protocols—focused on more efficiently ensuring product safety, demonstrating medical utility, and attaining mass-produced product. To provide concrete and specific recommendations, the FDA plans, in the short term, to create a “critical-path opportunities list” prioritizing the most pressing development problems and identifying areas most likely to experience quick improvements and have the largest public health benefits.

FDA “researchers have a unique vantage point on scientific challenges that cause delays and failures in product testing and manufacture,” McClellan says. The “opportunities list” is meant to be only the first broad step in an extensive process involving cooperation between the agency, industry, and academia. The biotechnology industry, at least, says it is ready for the challenge.

“This is a courageous statement from a government agency recognizing the serious problems that are preventing new, innovative drugs and biologics from getting to the patients who need them,” says Carl Feldbaum, president of the Biotechnology Industry Organization (BIO). “BIO is pleased to work with the FDA to develop those tools necessary to streamline and improve the product development process.”

—DAVID FILMORE

FDA: Increasing interaction, selectively

The FDA has selected at least seven drug candidates now in clinical testing for a new program designed to test the effectiveness of enhanced interaction between sponsors and the agency in improving the Investigational New Drug phase of development.

The Continuous Marketing Application (CMA) Pilot 2 program, mandated in the June 2002 reauthorization of the Prescription Drug User Fee Act, directs each of the agency’s drug review divisions to pick one product to receive continuous guidance and feedback from the FDA as it moves through its clinical track. Each candidate, according to the FDA, must already be designated a fast track product and should be selected on the basis of its potential public health impact and chances of benefiting from concentrated scientific and regulatory dialogue.

“We are honored to have been selected for inclusion into this exclusive and pioneering program,” says Hank Fuchs, president and CEO of IntraBiotics Pharmaceuticals. The FDA Division of Anti-Infective Drug Products chose the company’s lead product iseganan, an oral solution for the prevention of ventilator-associated pneumonia that recently entered an oral solution for the prevention of ventilator-associated pneumonia that recently entered a New Drug Application to the FDA for Surfaxin in April.

The agency indicates that two other products have also been chosen for the program. Nine divisions, which have yet to select candidates, will be taking applications until September 30.

The agency has, on a limited basis, engaged in early review of marketing applications in the past, but there has not previously been a formal program to assess the value and costs of such activities. Throughout its duration, an independent consultant will evaluate the CMA Pilot 2 program, along with a concurrent, less selective, and less interactive Pilot 1 program.

The precise nature of the feedback and interactions will be laid out in individual agreements between each drug sponsor and the FDA.

—DAVID FILMORE
Meeting for minds
Chemical Diversity Labs and the Laboratory for Drug Discovery in Neurodegeneration (LDDN) at Harvard Medical School are collaborating to discover new tools for understanding and treating neurodegenerative diseases, such as Alzheimer’s disease, Parkinson’s disease, ALS (Lou Gehrig’s disease), multiple sclerosis, and Huntington’s disease.

Under their agreement, Chemical Diversity will use its small-molecule collection and Chemosoft in silico software to select a maximally diverse set of compounds for biological screening against LDDN’s neurodegenerative disease targets. LDDN will in turn provide Chemical Diversity with screening data for creating second-generation libraries.

“Effective drugs for diseases of the central nervous system are especially difficult to develop, due to the complexity of the brain as well as its physical isolation by the blood–brain barrier,” says Ilya Okun, vice president of research and development at Chemical Diversity. “We see our collaboration with LDDN as an important source of de novo biological data to direct exploratory synthesis of chemical library systems for the central nervous system area.”

LDDN is a major initiative of the Harvard Center for Neurodegeneration and Repair, a decentralized community of Harvard Medical School-affiliated neuroscience researchers that has created what it calls a “drug discovery engine” comprising assay development, high-throughput screening, informatics, and exploratory medicinal chemistry.

“The goal of the LDDN is to transform basic neurobiological findings into drug discovery programs through the discovery of new chemical compounds that have promise as research tools or as novel therapeutics for neurodegenerative disorders,” says LDDN director Ross Stein. Establishing partnerships with biopharmaceutical companies is an important part of the Harvard group’s strategy to commercialize its drug candidates.

Financial terms of the agreement with Chemical Diversity were not disclosed. The current neurodegenerative drug market is approximately $30 billion.

—DAVID FILMORE

Proiling Parkinson’s
Per Andrén and colleagues at Uppsala University, Vanderbilt University, and the Karolinska Institute recently uncovered molecular events behind Parkinson’s disease by using matrix-assisted laser desorption/ionization (MALDI) MS. Unique MS protein profiles can be obtained in specific brain regions to study complex biochemical processes, the researchers say.

Parkinson’s is a common disease caused by selective degeneration of dopaminergic neurons in the brain. In the early stages of the disease, the administration of the dopamine precursor L-Dopa or dopamine receptor agonists can alleviate symptoms such as rigidity, imbalance, and tremor. However, the precise metabolic events in people with the condition are poorly understood. (J. Proteome Res. 2004, 2, 289–295).

The researchers mimicked human Parkinson’s in rats by administering the neurotoxin 6-hydroxydopamine (6-OHDA) to one side of the brain. They then sacrificed the animals and treated tissue sections for direct MS analysis. In a typical experiment, the scientists detected 200–300 protein and peptide signals ranging in size from 2 to 25 kDa. They found that these profiles were highly reproducible between consecutive brain sections and between equivalent regions of a single section.

The researchers compared the protein and peptide profiles of 6-OHDA-treated brains with and without L-Dopa administration. They found that although many MS peaks were similar between the two samples, there were distinct differences. For example, the level of a cytochrome c oxidase subunit, a metabolic marker of neuronal activity, was significantly lower in the dopamine-depleted brain than in the side treated with L-Dopa. Likewise, they noticed decreased levels of cytochrome c, an apoptosis-related marker, in non-L-Dopa-treated sections.

Aside from the identified markers, the researchers noted a variety of other peaks they are investigating. Similarly, they noted several changes in post-translational modification patterns between the samples, which might give some clue about the metabolic changes occurring in the dopamine-depleted and L-Dopa-treated samples.

“Left brain, right brain. Researchers performed direct MALDI MS analysis of brain sections to determine marker profiles of Parkinson’s disease. (Adapted with permission from Pierson, J.; et al. J. Proteome Res. 2004, 2, 289–295.)

“Our results showed that MALDI MS protein profiling directly on tissue sections provides unique information regarding the peptide and protein expression in experimental models of Parkinson’s disease,” the authors write. “The MALDI MS profiling may give a better understanding of the underlying pathophysiology.”

—RANDALL C. WILLIS
new and noteworthy

Delivering a nuclear payload

Researchers at the NIH, Ciphergen BioSystems, and Boston University have developed a radionuclide delivery system based on sequence-specific peptide nucleic acids (PNAs).

Although radiotherapy has played a significant role in treating diseases such as cancer, an inherent drawback is that it tends to destroy good cells as well as the bad ones. By targeting specific cancer-related genes, however, clinicians might be able to circumvent this problem.

Researchers can target gene sequences using an antisense approach, relying on sequence-specific oligonucleotides to deliver radionuclides such as $^{125}$I, $^{123}$I, or $^{111}$In. Unfortunately, oligonucleotides can be very sensitive to nuclease degradation. PNAs are more chemically and biologically stable than oligonucleotides because they carry a polyamide backbone instead of a sugar–phosphate backbone.

To assess the effectiveness of PNA delivery, Igor Panyutin of the NIH and his colleagues conjugated the metal-chelating reagent DTPA to free amino groups on a PNA and a DNA molecule, both with the same nucleotide sequences (Eur. J. Nucl. Med. Molec. Imaging 2004, in press). Using surface-enhanced laser desorption/ionization (SELDI) MS and HPLC, they noted reaction yields of >85%. The researchers then bound $^{111}$In to the conjugates.

Similarly, the scientists determined through DNA sequencing that the PNA complex produced a higher yield of target DNA breaks (5.35%) than the DNA complex (1.9%), and that, in both cases, the breaks occurred within the region bound by the PNA and DNA vehicles. Furthermore, by increasing the number of DTPA molecules conjugated to the PNA, they could increase the target break yield to almost 7%.

Although these studies were performed with only one PNA sequence, the researchers are confident the methods are applicable to a variety of target sequences. Similarly, they believe their in vitro results indicate in vivo possibilities.

“Recently, there has been significant progress in PNA cellular delivery that has substantially reduced the initial skepticism regarding the therapeutic and in vivo diagnostic use of PNAs,” the researchers write. “In addition, due to a neutral backbone, PNA appears to lack general toxicity and nonspecific protein binding.”

RANDALL C. WILLIS