Oral Vector Vaccines Target Biothreats

Since the 2001 terrorist attacks in the United States, U.S. governmental agencies have funded numerous multimillion-dollar biodefense projects. Among these projects are vaccine development programs aimed at biological agents such as plague, anthrax, tularemia, and smallpox. In particular, several research groups working on oral anthrax vaccines have made significant advances in their projects, with many slated to begin clinical trials this year.

For example, researchers at the University of Maryland’s Center for Vaccine Development (Baltimore, MD) are preparing a Phase I clinical trial of an oral “vector vaccine” carrier technology that enables anthrax antigens to be expressed in salmonella bacterium. The genes that encode the bacterial toxins are removed from the salmonella organism and the anthrax antigens are added. Then, when the tableted salmonella is swallowed and enters the gastrointestinal tract (GIT), it binds to and burrows into the GIT wall. However, unlike pathogenic salmonella, the attenuated form doesn’t initiate a severe inflammatory response, enter the blood stream, survive in the intestinal tissue, or cause illness. However, the vector vaccine does elicit a broad immune response.

"Results from preliminary human and animal studies suggest that by colonizing in the intestine and burrowing in before it self-destructs, the vector illicit a protective immune response without generating symptoms or adverse effects," says James Nataro, M D, PhD, primary investigator for the study and professor of pediatrics at the University of Maryland. Chief Scientific Officer Steve Chatfield, who is working on a similar drug delivery method called “spi-VEC” at Microscience (Berkshire, UK), adds, “Salmonella works as a good oral vaccine carrier because the body recognizes it as an invading organism, even though it can no longer cause disease.”

The oral form of the anthrax vaccine has several advantages over traditional, injectable products. According to Nataro, “Patients are less likely to have severe allergic reactions because the GIT filters out allergens.” The only anthrax vaccine currently available on the market (an injectable form) causes serious allergic reactions in one of every 100,000 doses.

Oral anthrax vaccines also require fewer doses than their injectable counterparts, which must be taken several times over a period of months. “We’re hoping the oral vaccine will achieve immunity in 1 or 2 doses,” says Chatfield.

In addition, the oral vector vaccines can be developed in multivariate form, meaning that one tablet can protect against more than one biological agent at once. For example, Avant Immunotherapeutics, Inc. (Needham, MA) is currently experimenting with the cholera and typhoid fever bacterium as its vectors of choice. The carriers are engineered with plague and anthrax genes to provide immunity to anthrax, plague, and cholera/typhoid. “The plague component of our typhoid-plague-anthrax vaccine is scheduled to enter a Phase I clinical trial before the end of the year,” says Una S. Ryan, PhD, Avant president and chief executive officer. Noting that providing for the rapid onset of immunity also is a goal, Ryan says their approach “is to make an effective, single-dose vaccine that protects rapidly within days, not weeks or months.”

Ease of production and delivery are also important considerations, says University of Maryland’s Nataro. “If there’s an outbreak, we should be able to vaccinate large amounts of people in a very short time frame, without a complicated and

“Rapid-release” Gives Fast Relief to Migraine Sufferers

Pharmaceutical companies have long sought ways to deliver faster relief to sufferers of one of the world’s most common neurological conditions—migraine headaches. The latest innovation in this effort is a reformulation of GlaxoSmithKline’s (GSK, Research Triangle Park, NC) “Imitrex” (sumatripan succinate) tablets to take advantage of the company’s new “rapid-release technology.” Rapid-release Imitrex tablets maintain the same route of administration as conventional tablets (swallowed whole with water), but increase the onset of action by enabling the tablets to dissolve more quickly once in the stomach. By swallowing the tablet, the active drug gets to the site of absorption more quickly than other routes such as oral melts that dissolve on the tongue and may not absorb as fast. “The act of swallowing is typically the fastest route to the stomach,” says Frederick Taylor, M D, a headache specialist and neurologist at Park Nicollet Health Services (St. Louis Park, MN), and an advisor for GSK. Taylor says the new formulation is intended to help overcome absorption problems associated with “gastric stasis,” the slow movement of the

Figure: The reformulated Imitrex tablets are packaged in a new punch-card configuration.

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New IMS Sample Injector Facilitates Cleaning Validation

To provide analysts a better method of introducing cleaning validation samples into their ion mobility spectrometry (IMS) systems, Smiths Detection (Warren, NJ) has added a high-performance injector (HPI) to its "Ionscan" line of IMS technologies. According to the company, the injector increases control over the sample-introduction conditions and improves the reproducibility of the results, making them comparable to the results achieved with the use of traditional high-performance liquid chromatography (HPLC) systems.

IMS detects and identifies compounds by measuring the mobility of ions in the gas phase. In a cleaning validation process, samples are taken using standard swabbing techniques, extracted into a solvent, and injected into the Ionscan using an autosampler.

With the system's original injection method, the sample is injected into a PTFE substrate. With the new HPI, the sample is injected into a glass capillary that can be configured for the needs of the application, under precisely controlled airflow and temperature conditions. "The HPI allows us to get reproducibility very similar to what is achieved with HPLC and offers the user a lot of flexibility," notes Robert Sandor, PhD, vice-president for life sciences at Smiths.

Analysis of the sample begins with thermal desorption to vaporize the sample. Once the sample is in the vapor phase, an airstream carries it into the ionization section of the instrument, where it is selectively ionized.

Selective ionization is conducted using atmospheric chemical ionization, in either negative or positive mode, using an appropriate dopant to provide the desired selectivity. "We don't generally fragment these compounds," notes Reno DeBono, PhD, director of research and development for life sciences at Smiths. "We work with molecular ions.

With IMS, compounds are identified on the basis of the speed at which they travel through a drift tube, which depends on their size and shape. Ions are gated into a drift tube with the use of an electric field with a gradient of 2000 V and with air flowing in the opposite direction at atmospheric pressure. When the ions reach the end of the tube, they strike a plate, producing an electric current, which is then measured. The strength and speed of the ions' passage through the drift tube is displayed in a plasmogram, very similar to a chromatogram. Typical drift times are between 10 and 20 ms. Accurate identification is based on the detection of peaks within ±0.04 ms of their expected peak positions.

The IMS method is useful for distinguishing compounds having similar properties and characteristics. Sandor cites the example of comparing hexane and benzene. Although they have a very similar molecular weight, benzene is stiff and pancake-shaped, and hexane is like a flexible wire. "Because of their different shapes, even if their molecular weights were identical, the IMS can distinguish them," notes Sandor. "We even have customers who have preliminary evidence that they can distinguish between diastereomers [molecules with two or more chiral centers]," adds DeBono.

Sandor says the sensitivity of IMS is similar or better than that of HPLC. "For HPLC, the best sensitivity I've aware of is about 0.2 ng," he says. Although the sensitivity of the Ionscan varies depending on the compounds involved, the system can detect quantities from 0.01 to 10 ng of many pharmaceutical compounds. In tests conducted with diazepam, the instrument was able to detect quantities as low as .004 ng.

The system does have limitations in terms of the types substances it can detect. "The compound has to be vaporizable and ionizable, and have a maximum molecular weight of about 1200," says Sandor, which means that the system cannot be used with large biopharmaceutical ingredients such as peptides. "As a general rule, the IMS can detect approximately 80% of the APIs we test it with," he concludes.

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Needle-Free Drug Delivery System Blasts Through Skin

Finding new ways to deliver drugs through the skin has always been a challenge. Researchers from the Harvard-Massachusetts Institute of Technology (MIT) Division of Health Sciences and Technology are developing a new technique that can painlessly blast medications through the skin, eliminating the need for hypodermic needles for drug delivery and blood analysis.

As explained by MIT's researchers, the technique, called "microscission," uses a stream of gas to bombard small areas of the skin with tiny (25 μm) crystals of inert aluminum oxide. James Weaver, PhD, biophysicist from the Harvard-MIT Division of Health Sciences and Technology, says, "The process removes the stratum corneum (outermost layer of skin) over a tiny area allowing for access to the viable epidermis."

The sharp particles remove the rough surface layer of the skin and create tiny holes, or microconduits, that lead into the lower layers of skin and serve as receptacles for drugs. The particles "scize" (cut) tissue, which is removed by the gas flow. The resulting microconduits formed are invisible to the naked eye, measuring less than ¼ mm in diameter and between ½ and ¾ mm in depth. The entire process takes less than 20 s.

The researchers have demonstrated "proof-of-principle" that microscission can be used for drug delivery and is much less painful than a needle prick. The technology continues on page 20.
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stomach commonly seen with migraine attacks. The reformulation doesn’t affect the way the active ingredient targets the nerves and blood vessels that cause pain, nausea, and sensitivity to sound and light, which are believed to be the factors that trigger the total migraine.

Rapid-release technology incorporates sodium bicarbonate into the drug formulation to help break apart the drug particles at a more rapid rate. According to Taylor, this delivers a dis-solution rate “five times that of a conventional tablet.” Officially, the company claims that rapid-release Imitrex tablets will dissolve within two minutes, but Taylor says the reality is actually faster.

A recent study indicated that two-thirds of patients treated with tablets using rapid-release technology claimed to be completely free of pain in less than two hours. “That’s a huge number compared to what you see with other tablets,” says Taylor. The results were based on a double-blinded, placebo-controlled clinical trial in which patients were instructed to take one 100-mg tablet within one hour of the start of a migraine headache.

According to GSK’s Manager of Product Communications, Robin Gaitens, the incorporation of rapid-release technology doesn’t impact the tablet manufacturing process or cause any challenges in redeveloping the formulation. However, the reformulated tablets, which are now hitting pharmacy shelves, are packaged in a new punch-out card configuration, replacing the original Imitrex peel-out packaging (see Figure 1). The new packaging is intended to give patients easier access to medication, avoiding the fumbling often associated with the use of a peel-back cover—an important consideration for patients already suffering from migraine symptoms such as lack of coordination and unclear thought processes.

According to the National Institute of Neurological Disorders and Stroke and the National Headache Foundation, migraine headaches affect nearly 30 million Americans, and it’s estimated that as many as 20 percent of the world’s population suffer from them.

-Doreen R. Coppola

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expensive manufacturing method.” Adds Microscience’s Chatfield, “For the Spi-Vec oral vaccines, it isn’t necessary to perform the complicated protein purification process that’s needed for injectable vaccines.”

Avant uses a classification or vitrification technology as an alternative to freeze-drying. “Our technology enables us to lower the cost of goods because we dry without the freeze,” says Ryan. Live carrier organisms are fermented and preserved in a sugar solution. The mixture is then dried at the stable glass transition temperature, thus transforming the sugar solution into a toffee-like glass. Avant’s data show that the process could be useful, however, as a painless way for people with diabetes to check their blood sugar levels.

Microscission may have multiple applications. For example, blood glucose measurements were taken with two commercial monitoring systems, and 180-μm deep microconduits yielded volumes of several μL, with a faint pricking sensation as blood entered tissue. Other testing has shown microconduits reduce through-skin electrical impedance between two ECG electrodes from 4000 to 500 Ω. In all applications tested to date, microscissioning has been shown to be painless, rapid, and free of scarring.

Although further studies are needed, researchers say microscission is a minimally-invasive drug delivery approach to those with “needle-phobia,” and could be set to revolutionize extraction of interstitial fluid and blood samples. The research team hopes to move the technology into initial clinical trials by the end of 2004.

-Megyn Bates

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Sandor says that 5 out of the top 10 largest pharmaceutical companies are using the system for cleaning validation in pilot-production facilities, and one in commercial production. “There are about 25 validated methods that have been reported back to us from our customers,” he says. Controlled studies are currently being conducted, the results of which are expected to be published later this year.

The main advantages for IMS cited by the company are its speed and simplicity of use. Typical throughput time for the IMS is 60-102 s, which is faster many standard HPLC methods that can take 10-20 min. This speed can reduce the length of equipment downtime that occurs between batches while cleaning validation is conducted.

-Laura Bush