Patch over pill?

Women suffering from severe symptoms of menopause may find an estrogen replacement skin patch far safer than the pill form, according to a recent study conducted in several hospitals in France.

Oral estrogen replacement therapy (ERT) has been reported to raise the risk of venous thrombosis (blood clots in the veins), but little is known about the effect of transdermal estrogen. For a period of six months, the research team randomly assigned 196 postmenopausal women to estrogen either in pill form or as a skin patch, both combined with oral progesterone, or inactive placebo treatment (*Arterioscler. Thromb. Vasc. Biol.* **2003,** *23,* 1671–1676).

"This randomized trial highlights the importance of the route of estrogen administration in prescribing postmenopausal hormone therapy," commented Emmanuel Oger, one of the doctors participating in the study, now at Dandenong Hospital in Victoria, Australia.

Because activated protein C (APC) resistance is a known risk factor for venous thrombosis, the researchers measured the effect of APC on the regulation of a clotting factor, thrombin, for each of the treatment groups. For women taking oral ERT, there was a statistically significant increase in APC resistance compared with those in the placebo and transdermal groups. But there was no significant difference in effect between the transdermal treatment and the placebo. Additionally, after six months, markers of blood coagulation activity were significantly higher in the oral estrogen group than in the transdermal or placebo groups.

"Taken together, these data provide a plausible biologic mechanism to the clearly demonstrated association between oral estrogen and venous thrombosis," wrote the researchers in the study. In addition, according to Oger, these findings join a "strong body of biological evidence that suggests a lower risk for venous thrombosis, if any, among users of the [estrogen] skin patch."

Elucidating the full mechanism of transdermal ERT drug delivery and assessing its overall safety profile require further investigation. Oger and colleagues hope their results will encourage such work.

—FELICIA M. WILLIS

PHOTO: PHOTODISC

Modeling medicine

Many researchers and clinicians expect that the wealth of bioinformatics data will one day allow scientists to predict the efficacy and side effects of a particular drug before the compound is ever tested on patients. Other researchers see this as merely a pipe dream, but based on the recent results of researchers in Israel and The Netherlands, that pipe dream is one step closer to becoming a reality (*Brit. J. Hematol.* **2003,** *123,* 683–692).

Patients undergoing hematological or cancer treatment can suffer from a condition known as thrombocytopenia, whereby the body cannot produce enough platelets for wound healing. One treatment for this problem involves administering recombinant or synthetic thrombopoietin (TPO), a cytokine that stimulates platelet production (thrombopoiesis). Unfortunately, under the current treatment regimens, exogenous TPO can cause immunogenic complications that limit its efficacy.

To address this issue, the researchers developed a mathematical model of thrombopoiesis, using data available in the medical literature that encompasses all of the biological pathways, from stem cells to peripheral platelets, as well as the pharmacokinetics and pharmacodynamics of circulating TPO. They then used this model to predict the efficacy and side effects of altered dosing regimens on mice and rhesus monkeys, testing their predictions in vivo.

The researchers divided mice into several groups, including those that received a single 17.5-μg/kg dose of TPO and another that received a total dose of 8 μg/kg in 4 injections given at 24-h intervals. The model predicted that the lower dosing regimen would provide the same benefits as the larger dose but without the side effects. This prediction was verified when the researchers examined in vivo platelet counts.

They then repeated the experiment with rhesus monkeys and found similar results for most of the animals, the one exception being an animal that became refractory to TPO treatment because of the production of anti-TPO antibodies. The researchers also found that if they adjusted their model for monkey-specific parameters, they could accurately predict the response of individual monkeys to treatment. The researchers did find, however, that in vivo changes in platelet production occurred earlier than was predicted by the model, suggesting that some adjustments to the model data were necessary.

Human testing is under way, but according to the researchers, "This tool is expected to be of aid in suggesting improved drug protocols for the individual or for the population of patients."

—RANDALL C. WILLIS

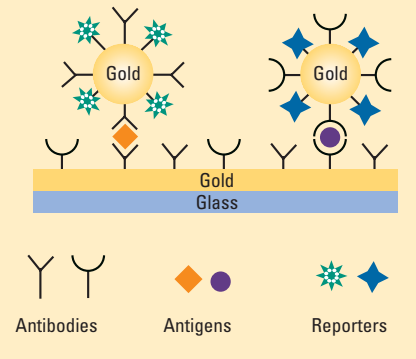
PSA the Raman way

Since 1988, prostate-specific antigen (PSA) has been used as an assay for prostate cancer, the second-leading cause of cancer deaths in adult males in the United States. PSA is a 33-kDa protein with normal blood plasma levels of 4–10 ng/L. The predominant form of the protein in serum is a complex with α_1-antichymotrypsin. Lesser amounts of PSA are bound to two other proteins.

Current assays detect unique epitopes in each complex. A decrease in free PSA compared to complexed PSA indicates a higher probability of cancer. However, the levels of recurring PSA in the initial stages of cancer are usually too low for current assays to detect.

As part of their effort to develop a more sensitive assay, Marc Porter and colleagues at Iowa State University (*www.iastate.edu*) recently reported on a surface-enhanced Raman scattering (SERS) immunoassay capable of detecting femtomolar concentrations of PSA (*Anal. Chem.* **2003,** *75,* 5936–5943). Gold nanoparticles were coated with a monolayer of a material, 5,5′-dithiobis (succinimidyl-2-nitrobenzoate) (DSNB), that exhibits a strong Raman scattering signal. These labels were designed to be bifunctional—containing disulfides for chemisorption to the gold nanoparticle surface and succinimides for coupling to the antibodies.

The assay follows a typical sandwich model. A glass chip was fabricated with the appropriate antibodies attached. Antigens were added to bind to the antibodies. Then the so-called SERS reagent—the antibody- and DSNB-coated gold complex—was added. This created an amplified signaling system for a Raman spectroscopy assay. The labeling protocol, in which the Raman reporter tag (DSNB) forms the chemical bridge between nanoparticle and antibody, allows for a close proximity of the metal to the reporter and maximizes the surface area of the reporter, both of which contribute to an increased amplification.



SERS capture. Gold nanoparticle immunoassay. (Adapted with permission from Grubisha, D. S.; et al. *Anal. Chem.* **2003,** *75,* 5936–5943.)

Free PSA could be detected down to 1 pg/mL in human serum and 4 pg/mL in bovine serum albumin within 60 s. And, say the researchers, the assay has strong potential for multiplex analysis: Because Raman bandwidths are 10–100 times narrower than typical fluorescent immunoassays, it allows long-wavelength excitation of multiple labels with a single excitation source and is much less susceptible to photobleaching and quenching than fluorescence. Work is under way to develop an assay for the concurrent determination of free and total PSA, for even more reliable prostate cancer diagnosis.

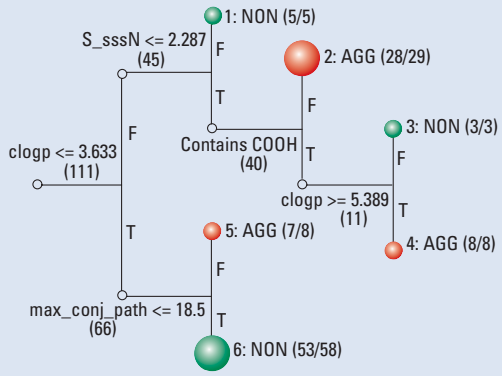
—MARK S. LESNEY

Promiscuous pharma

A good drug is a target-specific drug. Molecules with many potential protein partners are supposed to be left in the early-stage high-throughput screening waste bin. But on occasion, this does not occur, and screening hits are later found out to be promiscuous false positives. Brian Shoichet and colleagues from the University of California, San Francisco (*www.ucsf.edu*), Northwestern University (*www.northwestern.edu*), and Pfizer (*www.pfizer.com*) have one possible explanation for this: that some hits form molecular aggregates in solution that are promiscuous inhibitors. In a recent study (*J. Med. Chem.* **2003,** *46,* 4477–4486), the researchers screened 50 known drug compounds against 3 enzymes that recognize dissimilar ligands and are not considered targets of any of the drugs. To be judged a promiscuous aggregate-based inhibitor, a compound had to inhibit all three enzymes in a time-dependent manner and be sensitive to detergent and enzyme concentration. Furthermore, it had to form particles that were detectable by light scattering. Of the 50 compounds tested, 4 unambiguously fit the criteria and 3 fit it only at high concentrations.

The researchers then analyzed another set of 111 compounds, 47 of which were known aggregators and 64 that did not aggregate even at micromolar concentrations. In their initial analysis, they determined that either solubility or hydrophobicity (clogP) provided some degree of distinction between the two groups but that neither criterion alone was sufficient.

The scientists generated a decision tree and used a simple determination of percent of categories correctly predicted as a method to assess the validity of each decision. They achieved a model that correctly placed 93.7% of the compounds using only four criteria: clogP, an electropological state (S_sssN), degree of compound conjugation (max_conj_path), and the presence of a carboxylate group (COOH).



Decisions, decisions, decisions. Using recursive partitioning analysis, researchers divided a library of 111 compounds into families that were nonaggregating (NON) or aggregating (AGG). (Adapted with permission from Seidler, J.; et al. *J. Med. Chem.* **2003,** *46,* 4477–4486.)

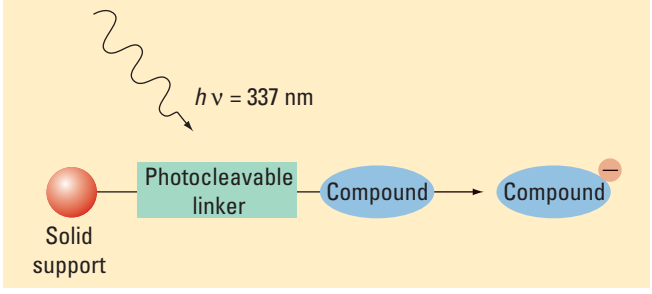
The scientists expressed concern that several approved drug compounds develop into promiscuous aggregates. They welcome larger studies of other pharmaceutical compound libraries "to investigate just how common such molecules are."

—RANDALL C. WILLIS

Split-pool mass spec

MS offers the potential for unambiguous compound identification that is not possible with FTIR and NMR spectroscopy due to their widespread signal overlap. However, in solid-phase synthesis, cleaving the product from the resin bead has typically been a prerequisite for MS analysis. In recent work, the feasibility of performing the cleavage inside a MALDI spectrometer to accomplish ionization has been demonstrated, but it incorporates matrix elements that hinder routine detection of the low-molecular-weight molecules (<500 Da) that dominate medicinal chemistry. Researchers at Universität Dortmund (*www.uni-dortmund.de*) in Germany, however, have developed a matrix-free on-bead monitoring MS method (*J. Combi. Chem.* **2003,** *5,* 814–820).

They used a simple photolabile compound as a linkage between the solid support and the organic compounds of interest. The two different linker groups that were studied—phenacyl and *o*-nitroveratryl—have absorption spectra that readily overlap with the 337-nm laser emission that is standard fare in commercial MALDI-TOF spectrometers. Upon laser excitation, it was expected that the linker would be cleaved, releasing a negative analyte with sufficient energy to be in the gas phase for straightforward MS analysis (see figure).



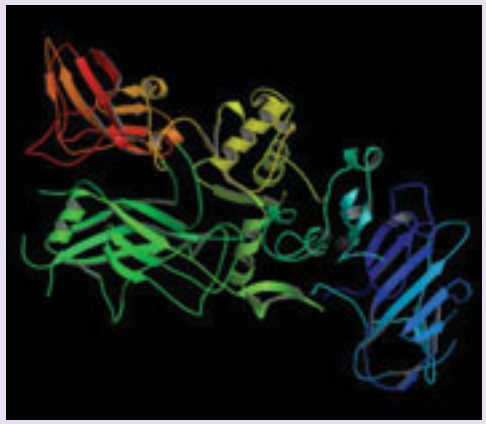
Broken link. Photocleavable linkers can be used for direct MS monitoring of solid-phase organic synthesis (Adapted with permission from Gerdes, J. M.; Waldmann, H. *J. Combi. Chem.* **2003,** *5,* 814–820.)

In initial studies, 19 of 24 carboxylic acid compounds that were linked to a resin via phenacyl were correctly detected with this approach. Four of the compounds that showed negative results have pronounced photoreactivity of their own that competes with the photocleavage reaction, pointing to an important limitation of this approach.

The scientists tested the procedure with three different solid-phase reactions—synthesis of 1,4-benzodiazepines, Pd-catalyzed synthesis of biaryls, and a two-step oxidation-Grignard reaction—in which the reactions were coupled to the resins via a photolabile linker. The expected products were detected in all cases by direct MS analysis of the beads.

According to the study report, the high sensitivity of MS and the high precision of lasers indicate that MALDI-TOF MS "could be employed very advantageously to deconvolute compound libraries generated in split-pool synthesis."

—DAVID FILMORE



Anthrax protective antigen.

STRUCTURE: PROTEIN DATA BANK

Anthrax vaccine contract

The U.S. government has greatly increased its funding of R&D for new and improved antibioterrorism technologies. In response, private industry in the United States and abroad is gearing up to serve this expanding public sector market. An illustration of this is the recent successes of VaxGen (*www.vaxgen.com*) with its anthrax vaccine candidate, rPA102.

The anthrax letter attacks that occurred in the United States in 2001 put particular attention on the *Bacillus anthracis* pathogen. And since the completion of the mapping of the anthrax proteins that allow the disease to attack humans and other animals, researchers have come closer to developing treatments.

Recently, the U.S. National Institute of Allergy and Infectious Diseases (NIAID, *www.niaid.nih.gov/default.htm*) awarded an $80.3 million contract to VaxGen for the advanced development of rPA102. The vaccine, pioneered at the U.S. Army Medical Research Institute of Infectious Diseases (*www.usamriid.army.mil*), consists of an alum adjuvant and recombinant protective antigen (a genetically engineered version of a key anthrax protein) that is designed to induce antibodies that neutralize anthrax toxins.

The product was developed, according to VaxGen, to improve the safety profile and the dosing schedule compared with the only currently licensed anthrax vaccine in the United States, BioThrax (*www.bioport.com*). BioThrax requires 6 doses over 18 months; it is hoped that rPA102 will require only 3 doses.

Accelerated development of the vaccine by VaxGen began in the fall of 2002 under a previous $13.6 million NIAID contract. The most recent funding is targeted at animal studies, Phase II clinical trials, the scale-up and validation of the manufacturing process, and the production of 3 million doses of finished product. Perhaps most importantly for the company, the new contract strongly signals the government's interest in purchasing stockpiles of rPA102 from VaxGen with funds earmarked for the purchase, maintenance, and replenishment of anthrax vaccine by the pending federal legislation known as Project BioShield.

The vaccine's efficacy will be determined by animal testing based on the U.S. FDA's two-animal rule model, instead of the typically required large Phase III studies in humans. However, the stockpile purchase could take place before the FDA officially approves the drug for commercial use.

—KIMBERLY S. CLEAVES

Surrogate signs for SLE?

How does one measure therapeutic efficacy in a clinical trial for a disease with varied manifestations that might involve inflammatory attack of any of a host of human organs and do not have a common biological explanation?

A draft guidance produced by the FDA and recently discussed by the agency's Arthritis Advisory Committee (*www.fdaadvisorycommittee.com*) attempts to answer this question for systemic lupus erythematosus (otherwise known as SLE or lupus), an autoimmune disease that affects millions of people—predominantly women—worldwide and has not seen a new drug on the market in about 40 years.

An important component of the draft discussed by the committee was the use of surrogate biomarkers. A reduction in a disease-related phenotype or improvements in survival are the traditional standards for drug approval, but when it is unclear what phenotypes are most indicative of a change in disease state, laboratory markers strongly associated with disease progression might be used as substitute end points. However, the suitability of this approach for lupus has been in question for some time.

Anti-double-stranded (ds) DNA antibody, which has been shown to be specific to lupus patients (although it is detected only in a subset of the overall patient population), received particular attention as a surrogate end point. This is significant because La Jolla Pharmaceutical Co. (*www.ljpc.com*) is pushing forward with Riquent, a drug candidate targeted at lupus nephritis.

The most recent trials for Riquent failed to show significant results for the trial's primary end point, delaying time to renal flare-up. However, it did show significant reductions in anti-ds DNA antibody. Further analysis indicated that for patients who showed a sustained reduction in this antibody, there was a notably greater delay in renal flare-ups and an improved quality of life compared with patients who did not show a sustained reduction. The company plans to use this data in its New Drug Application as an argument for applying anti-ds DNA antibody as an end point.

The advisory committee asserted that more evidence would be necessary before anti-ds DNA antibody could act as a sole end point, but it could be useful in combination with a disease activity index, health-related quality-of-life measures, and additional surrogate markers.

How this policy would affect the Riquent approval process still warrants discussion, but it is hoped that the final guidance will generally encourage greater activity in lupus drug development.

—DAVID FILMORE

KEY TERMS: assays and screening (pp 12, 15), clinical (pp 11, 15, 16), combichem (p 15), drug delivery (p 11), informatics (p 11), medicinal chemistry (pp 12, 14), modeling (p 11), money (p 15)

Malarial prophylaxis problem

The current chemoprophylaxis regimens for travelers to malaria-infected regions are not sufficient for an important subset of cases, namely delayed-onset malaria, according to researchers from Tel Aviv University (*www.tau.ac.il*), the U.S. Centers for Disease Control and Prevention (*www.cdc.gov*), and Emory University (*www.emory.edu*).

The scientists examined malaria surveillance data in both Israel (1994–1999) and the United States (1992–1998) (*N. Engl. J. Med.* **2003,** *349,* 1510–1526). They found 300 cases of malaria in returning travelers in Israel and 2822 cases in returning travelers in the United States.

For patients in whom infection was detected within 2 months after return (about 55% in Israel and 65% in the United States), the situation was predictable—about 90% of so-called early-onset Israelis and 80% of early-onset U.S. travelers had not used or ineffectively used malaria drugs. But for those in whom infection was detected after 2 months, the results were more troubling. About 80% of late-onset infected Israeli travelers (108) and more than 60% of such U.S. travelers (614) had used what were considered effective prophylaxis prescribed by physicians.

An important difference between the two groups was the infecting species. A large majority of the early-onset travelers were infected with *Plasmodium falciparum*, whereas most of the late-onset group had infections due to *P. vivax* and *P. ovale*.

In the initial liver stage of their life cycle in humans, the parasites enter and multiply in the hepatocytes, eventually causing these cells to rupture. Most malaria prophylaxis (e.g., chloroquine and mefloquine) targets the second stage, in which the multiplied parasites emerge to attack the red blood cells, causing clinical illness. A month of taking these drugs after a trip is highly effective for *P. falciparum*, the most malignant species, which progresses to the blood stage within a week of infection. But the liver stages of *P. vivax* and *P. ovale*, common in Ethiopia, Somalia, and many other areas outside sub-Saharan Africa, are known to persist for months or even years.

This data points to the need for drugs that target the liver phase of malaria parasites. There are several main candidates at this point, although sufficient clinical data is still lacking. Further discovery and development of liver-phase prophylaxes, say the researchers, would provide more fail-proof protection that could be used against all *Plasmodium* species. And, importantly, these medicines could theoretically be discontinued soon after a traveler departs an endemic area—a plus for improving patient compliance.

—DAVID FILMORE ■

PATIENT: PHOTODISC; MOSQUITO: CENTERS FOR DISEASE CONTROL AND PREVENTION