

IN BETWEEN THE LIGHT AND THE DARK

Developments in Photosensitive Pharmaceuticals

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Researchers are advancing the field of photodynamic therapy with effective drugs, new light sources, and improved drug delivery techniques for deep tissue penetration, site-specific treatments, and reduced side effects.

Many pharmaceutical manufacturers cringe at the thought of placing a photosensitive drug directly in front of a light source. But for years, drug developers have exploited the therapeutic effects of light-absorbing pharmaceutical compounds.

Although light-activated drugs are well-established treatments for diseases such as cancer, formulators now are expanding the boundaries of photodynamic therapy (PDT) to offer effective treatments for oncologic, ophthalmic, and dermatologic diseases. Recent advances in the technology—such as improved targeting of specific areas in the body and reduced side effects—are lengthening the list of potential applications for PDT.

Certain photoactivated drugs have specific production requirements and manufacturers must take care to avoid unwanted light in their facilities. Whether product degradation is caused by window-filtered light or inspection station lamps, facilities must be analyzed and monitored for places where light exposure could induce unwanted changes in a drug.

Light wanted

Traditionally used by oncologists, PDT is a binary treatment composed of two elements: a laser light source and a photosensitizing drug that absorbs light. Separately, neither component can produce therapeutic effects, but together, the synergy can destroy malignant tissue at a local treatment site.

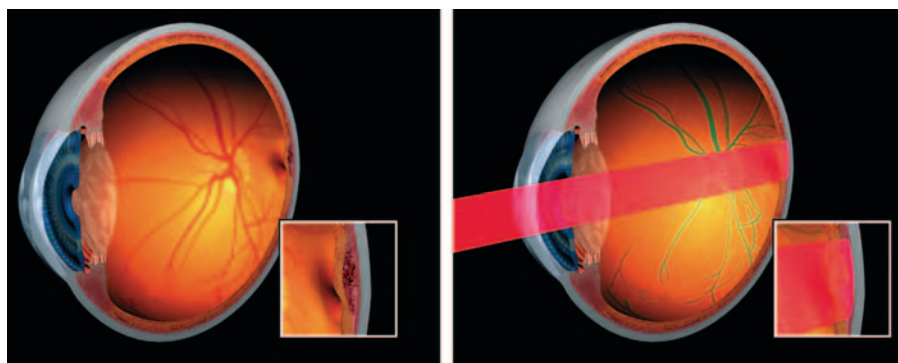
Upon administration to patients (traditionally *via* intravenous infusion), the photosensitizing drug is selectively retained in the neoplastic (cancerous) tissue. When exposed to a light source at certain visible wavelengths, the photosensitive

agent is activated and converts the light into energy through a photochemical process. The conversion generates an activated oxygen species, called singlet oxygen, which oxidizes critical elements of the cells. “This reaction can oxidize many biological molecules and structures,” says John Hill, MD, director of medical research at Miravant Medical Technologies (Santa Barbara, CA, www.miravant.com). “Because the activated oxygen species are so reactive, they have their effect in the immediate vicinity and do not travel far from where they are generated by the activating light. Thus, where the drug is localized and the light is applied are key determinants of where the end processes will take place.” The effect ultimately kills cancer cells and tumors and damages the abnormal blood vessels (neovessels) that nurture tumors.

Combatting limitations. Temporary blindness. Despite its great potential as a treatment for cancerous tumors, ophthalmologic diseases, cardiovascular diseases, and skin disorders, PDT is not without limitations. For example, typical light-activated treatments for age-related macular degeneration (a condition in which abnormal, leaky blood vessels in the retina lead to rapid vision loss) can cause acute post-treatment blindness, which in some cases isn’t reversible.

At present, Miravant Medical Technologies is developing a light-activated therapy to treat diseases at the back of the eye without causing this side effect. Although cancer treatments destroy the tumor, tissue, and supporting neovessels, therapies for macular degeneration “shut off” leaky neovessels without damaging surrounding tissue. The company’s “Photrex” (SnET2) therapy, which is in Phase III clinical trials, is administered intravenously and 10–15 minutes later, light is applied to the leaky blood vessels at the back of the eye using a laser-incorporated ocular slit lamp.

The site(s) within a cell where the photosensitizing drug localizes can be critical for determining the severity of damage the drug causes. Photrex localizes in mitochondria and initiates an apoptotic mode of cell death in the endothelial cells lining the neovessels. This leads to neovascular destruction via a gentle process in which cells collapse in on themselves without rupture or inflam-



MIRAVANT MEDICAL TECHNOLOGIES

Miravant's light-activated drug, Photrex, in combination with a low-energy laser, may destroy abnormal blood vessels beneath the macula.

mation. "The negligible rate of immediate post-treatment vision loss that we've observed in more than 720 patients could be caused by this noninflammatory mode of action and resultant lack of fluid buildup following treatment," says Hill.

To increase the drug's solubility, Photrex is formulated in a lipid emulsion that resembles a nutritional i.v. supplement. Similar macular degeneration drugs already approved for use are supplied in a lyophilized form that must be reconstituted and used within four hours. "Our drug is supplied ready for i.v. infusion and the shelf-life is approximately three years," notes Hill.

Extreme skin sensitivity. One common side effect of PDT is skin photosensitivity. Because the free drug accumulates in the skin and eyes after treatment, some patients experience post-treatment skin sensitivity for as long as 6 weeks. Most drugs are activated by visible light, and therefore normal sunscreen—which only guards against ultraviolet (UV) light—won't protect patients against rash and sunburn. Says Miravant's Hill, "Skin photosensitivity varies from drug to drug, but it's almost impossible to have a drug that's activated by light as part of your therapy without also having some potential for sun sensitivity."

To address this problem, researchers at the University of Buffalo (UB, Buffalo, NY, www.buffalo.edu) have developed a nanoparticle drug delivery system that may help eliminate skin photosensitivity. Ceramic-based nanoparticles—made with a class of inert, nontoxic, and non-immunogenic materials called organically modified silica—encapsulate and form a permeable membrane around the hydrophobic photosensitizers. This coating creates a water-compatible shell that enables the drug to be dispersed more readily and prevents its self-aggregation and loss of fluorescence.

Once nanoencapsulated, the drug remains inside the particles. "Because photosensitizers are not released from the particles [into the bloodstream], they are less likely to accumulate in the skin and eyes, thereby reducing phototoxic side effects," notes Paras Prasad, PhD, executive director of the Institute for Laser, Photonics, and Biophotonics and distinguished professor of chemistry at UB. The nanoparticles, which have a 30-nm diameter, are re-

tained only by neoplastic tissue because macrophages cannot capture them. Because oxygen must be present in the cellular area to activate the drug, the nanoparticles have 0.5–1-nm pores that allow oxygen to diffuse through freely.

Although the particles have only been synthesized for research applications so far, the research team does not anticipate facing major problems for scaling up to production level. "The aqueous dispersions are stable and for some applications, they can be dried and stored for extended periods of time," says Prasad.

Other companies are using next-generation compounds and lowering their recommended doses to limit skin photosensitivity. "Historically, people were exposed to much higher doses of these drugs than the doses we use today," says Miravant's Hill. "The second generation compounds in use today produce some residual photosensitivity, but it's very manageable."

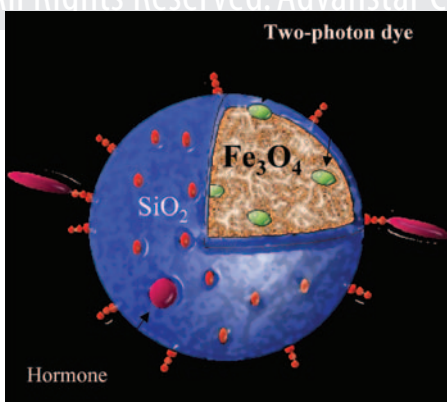
Says Albert Luderer, PhD, president and CEO of Light Sciences Corporation (Snoqualmie, WA, www.lightsciences.com) of the company's "LS11" light-activated drug, "We ask that people restrict their sun exposure for 48 hours, but we haven't seen any other significant side effects. We use an extremely benign drug." The drug has a plant source that the Light Sciences team synthetically modifies for use. "The body doesn't mind the drug," says Luderer. "And it's not metabolized. About 98% of the drug is collected by the liver and then it's excreted."

In addition, says Stuart L. Marcus, MD, PhD, chief scientific and medical officer of DUSA Pharmaceuticals Inc. (Wilmington, MA, www.dusapharma.com), other drug delivery methods can be used to reduce light sensitivity. "We offer topical drugs, which don't give you a full-scale skin photosensitivity," says Marcus. "If you protect the area from sunlight, you don't have to worry about it."

Only small and shallow tumors. Light in the visible wavelength typically required for photoactivation scatters before large, deep tumors can be reached. Although tissue penetration of 5–6 mm deep has been reported, in practice, this depth has not been observed consistently.

Another potential advantage of UB's nanoencapsulation technology is to enable the delivery of new formulations and photosensitizers that can be activated by

longer wavelengths of light. The use of upconverting nanophosphors, which can be excited by a continuous laser at long, low-intensity wavelengths, is the key to deep penetration, according to the research team. The process enables two-photon absorption and produces illuminance by upconverting infrared frequencies to visible light. According to Prasad, "Infrared light in this spectral range penetrates deeper into tissues than visible or UV light."



The University of Buffalo's nanoparticles encapsulate photosensitizers for deep tissue penetration and reduced side effects.

In addition, the upconverting nanophosphors can target larger tumors. "We are also investigating the use of biotargeting to selectively attack specific cells or to localize the photosensitizer in specific organelles to increase the effectiveness of the drug action," notes Prasad. The UB researchers recently received a \$925,000 grant from the John R. Oisehi Foundation to continue the development of their nanomedicine and nanobiotechnology.

The use of chlorophyll as a photosensitizer could be another way to attack large tumors. Derivatives from chlorophyll, the green pigment that allows plants to capture sunlight, can absorb light at 760 nm. "This is practically the best window for light penetration in humans because it's the best compromise between the light absorption of hemoglobin and other pigments," says Avigdor Scherz, professor in the Weizmann Institute of Science's plants and sciences department (Rehovot, Israel, www.weizmann.ac.il). The base of many photoactivated therapies is a red pigment derived from hemoglobin that must be activated by red light. Not only is the light's tissue penetration shallow, but the photosensitizers require oxygen for activation. Because deep, bulky tumors are not oxygen-rich, typical photosensitizers can only attack small, flat growths.

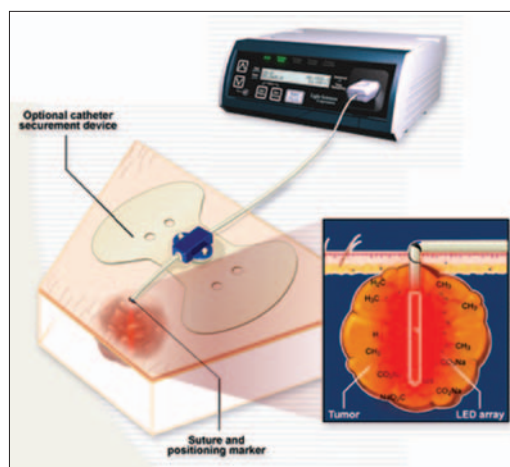
Researchers at the Weizmann Institute believe chlorophyll derivatives can kill larger tumors because they can absorb the near-infrared light that enables deep penetration. Chlorophyll also can be activated in low-oxygen conditions. "Chlorophyll is meant by nature to harvest solar energy, and therefore provides huge absorption in the visible and the near-infrared wavelengths," says Scherz.

Rather than relying on tumor uptake, the drugs evoke a specific response from tumor blood vessels that cut off its blood supply. Because the chlorophyll photosensitizer remains in the vasculature, the drugs have half-lives of only 10 minutes. Researchers believe this reduces skin photosensitivity and makes the treatment repeatable within a short period of time.

At present, the Institute is conducting Phase II clinical trials on a chlorophyll-derived treatment for prostate cancer patients who failed radiation therapy. According to Scherz, preliminary results from the study show good efficacy of the treatment. Clinical trials for a drug to treat macular degeneration will begin within the next few months.

Preventative applications. Finding a way to prevent cancer has been a topic of interest for years. According to researchers in the field, PDT may prevent certain types of cancerous tumors. "PDT is an amazing therapy that has the potential to be useful for so many things, from preventing blindness to treating skin cancers," says DUSA Pharmaceuticals' Marcus. "I think the future of PDT may be preventing the recurrence of cancer in patients that have had surgery for colon cancer or other types of cancer."

DUSA is already testing its patented "Levulan" drug as a means to prevent the recurrence of tumors in patients who have basal cell carcinoma. In some individuals, a genetic defect may cause



Light Sciences' prolonged photoactivation technique for destroying cancerous tumors.

dozens of cancerous growths annually; patients must undergo several surgeries per year to remove tumors. In a recent study, patients treated with a blue light-activated Levulan treatment did not develop skin cancers for 8 months in the areas treated with the drug. According to Marcus, this treatment could have broader implications as a preventative skin cancer therapy. "If you can keep cancer away from these patients, you might be able to prevent it in the general population," he explains. The company currently is investigating whether patients treated with Levulan develop fewer skin cancers over longer periods of time.

New laser lights. To a certain extent, the efficiency of PDT depends on the light source. "If you don't have the right way to activate the drug, it won't be an effective therapy," says Light Sciences' Luderer. Depending on the application, light sources for PDT have included lasers, light-emitting diodes (LED), and fluorescent light using delivery systems such as fiber optics, catheters, or endoscopes. But as the field of PDT develops, pharmaceutical companies are experimenting with new ways to make their light sources more effective.

Conventional laser-light sources for PDT deliver intense light for seconds or minutes, but Light Sciences has developed a light source that provides prolonged photoactivation. According to the company, this technique enables the delivery of higher light doses, amplifies the intensity of the light wave, and increases a drug's "killing capacity" of cancerous tumors.

A catheter-like device with flexible LED arrays is implanted into tumors using a percutaneous procedure. Drug is delivered over several hours. The constant, low-energy light will preserve the drug so that it recycles over and over again. This process creates an overwhelming number of singlet oxygen molecules, but does not compromise the drug's strength. "By doing this, you have a winning combination," says Luderer. "When you treat over a prolonged period of time, recycle the drug, and create more of the singlet oxygen, you get the maximum killing

Advances in photostability chambers

In recent years, manufacturers of photostability chambers have made strides in controlling light uniformity for researchers who require controlled environmental chambers for testing and storage. "Gone are the days of building a light box, inserting a light, and saying 'I'm doing my test now,'" says Bob Dotterer, applications engineer at Caron Products (Marietta, OH, www.caronproducts.com). "We've recognized that there are a lot of factors that must be controlled. Uniformity of light, humidity, and temperature are very important tools. This need has created a migration to higher quality products."

Caron has developed a line of photostability chambers that can be programmed to run for certain exposure levels in physical units that are consistent with guidelines established by the International Conference on Harmonization. This programming feature replaces the need for a chemical actinometric system and is superior to timers.

Light unwanted: manufacturing and packaging challenges

Photosensitive pharmaceuticals (including photoactivated drugs) must be shielded from light during the production process to protect against degradation. Photosensitizing chemicals usually have low molecular weights (100–500) and are planar, tricyclic, or polycyclic with resonating structures. “Certainly you want to manufacture in an area that is free of windows or has covered windows to prevent UV or UV-filtered light from affecting drugs,” notes Allen C. Templeton, PhD, research fellow at Merck (White House Station, NJ, www.merck.com). Window films, shades, or blinds usually are sufficient to block light.

Technicians who work in windowless facilities also must be cautious with their lamps. According to Templeton, the wavelength of each lamp should be monitored to ensure it doesn’t damage or change the drug in any way. “If there is substantial overlighting, a sleeve or cover can be used over the bulb,” he suggests. Because it is unsafe and difficult to operate in a manufacturing plant that is too dark, however, amber or red lighting conditions can be used.

Although it is frequently overlooked as a light-exposure risk, analytical testing also can cause degradation. Samples frequently sit in well-lit areas before technicians have time to analyze them. “When we handle samples, light exposure is critical,” says Steven W. Baertschi, senior research advisor at Eli Lilly and Company (Indianapolis, IN, www.lilly.com). “When you weigh, dilute, and prepare samples for analysis, they’re exposed to laboratory and window-filtered light.” In addition to covering lamps, samples in solutions should be prepared in light-protective volumetric flasks.

One must also examine any area where the drug is held for a long period of time. Transporting solutions for parenteral drugs from a compounding vessel into

a filling line, for example, can be a source of light degradation. Although the vessel usually is dark, the plastic tubing that transfers material from vessels into the fill lines could be a potential source of light exposure. “If the tubing is made of a clear material, you must consider how much light is getting through,” says Templeton.

Manufacturers should also minimize the time that vials are held in any one location without protection such as in conveyor belt holding stations, repackaging areas, or inspection stations. Striking a balance between the inspectors’ need for light and the drugs’ need for darkness can be a challenge. “You need to minimize the time the vials are in that station, but you also need to make sure the inspectors have enough time to do a good job,” Templeton stresses.

Templeton and coworkers have developed the concept of a “light budget” to determine how much light exposure a particular sample can undergo without changing. This strategy also can be used to estimate the costs associated with light exposure during manufacturing, packaging, and storage. Templeton and coworkers plan to present this topic in an upcoming paper.

Using bottles that block light from drugs is standard practice, but proper labeling may be the only way to ensure patients and physicians are aware of a drug’s photosensitivity. For example, patients who repack drugs in daily pill dispensers could unknowingly introduce light to photosensitive drugs. Or, nurses and physicians may not realize that some drugs in intravenous solutions degrade within 5 minutes. Says Templeton, “There needs to be better labeling. It may require greater flexibility from the regulatory bodies about what pharmaceutical companies can put on labels about photosensitivity.”

potential of these tumors.” In addition, bulky masses with diameters as large as 5×5 cm can be treated.

DUSA Pharmaceuticals is coupling its Levulan treatment with a new sheath light source to treat esophageal cancer. Typical light sources for reaching hollow organs involve a multistage procedure using an upper gastrointestinal tract endoscope. “This technique uses three insertions of the endoscope, which is uncomfortable for the patient and time-consuming for the doctor,” says Marcus. DUSA has developed a patented, proprietary device that covers the endoscope and enables the doctor to see through the device while it’s being used. The procedure only requires one insertion and is much quicker than traditional techniques. Notes Marcus, “It’s much more precise for light placement and it’s better for the treatment. We believe this is an advance to light delivery for light-activated therapies.”

Photochemical internalization

Light-activated compounds are also being studied as a gene therapy delivery system using nonviral and adenoviral delivery vectors. “With traditional PDT, the only thing you’re doing is killing the cells,” says Anders Högset, PhD, research director at PCI Biotech AS (Oslo, Norway, www.pcibiotech.com). “But light-activated molecules can be used for other things.”

Traditional macromolecular drugs cannot penetrate the cell membrane (e.g., of tumor cells) and are taken into the cell by encapsulation. Once inside the cells, the drug molecules are ineffective because they are degraded by the endosome. Photochemical internalization degrades the endosomal membrane so that the drug can escape and take effect.

Photosensitizers are incorporated into the membranes of endosomes that contain the drug. When light is applied at a wave-

length appropriate for the photosensitizer, the singlet oxygen produced destroys the endosome membrane and releases the drug molecules into the cell’s cytosol. For gene therapy, the DNA that is endocytosed makes the cell behave in specific ways. “We can make cells take up DNA so that they produce the effect you want,” says Högset. “If you want a cancer therapy, for example, we don’t kill cells with only the light effect. You can make the treatment much more specific with drugs that are specific for killing cancer cells without killing the surrounding normal cells.” The therapy is achieved by site-directed drug delivery induced by illumination of only the tumor.

PCI Biotech also is experimenting with other applications for its technology. For example, the company is using cytotoxic anti-cancer agents to make chemotherapy treatments that are already on the market more effective and specific. With such agents, the company can target tumors more specifically. Says Högset, “It can be very useful because you can kill cancer cells in a more specific way than you’re able to do now with chemotherapy or radiation.” In addition, researchers are experimenting by coupling antibodies with cytotoxic agents that can then be activated by illumination once the tumor takes them up. The company plans to begin clinical trials with its photochemical internalization treatments within 18 months. **PT**

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