New Light on Medicine

Pigments that turn caustic on exposure to light can fight cancer, blindness and heart disease. Their light-induced toxicity may also help explain the origin of vampire tales

By Nick Lane

Stories of vampires date back thousands of years. Our modern concept stems from Bram Stoker's quirky classic *Dracula* and Hollywood's Bela Lugosi—the romantic, sexually charged, bloodsucking outcast with a fatal susceptibility to sunlight and an abhorrence of garlic and crosses. In contrast, vampires of folklore cut a pathetic figure and were also known as the undead. In searching for some underlying truth in vampire stories, researchers have speculated that the tales may have been inspired by real people who suffered from a rare blood disease, porphyria. And in seeking treatments for this disorder, scientists have stumbled on a new way to attack other, more common serious ills.

Porphyria is actually a collection of related diseases in which pigments called porphyrins accumulate in the skin, bones and teeth. Many porphyrins are benign in the dark but are transformed by sunlight into caustic, flesh-eating toxins. Without treatment, the worst forms of the disease (such as congenital erythropoietic porphyria) can be grotesque, ultimately exacting the kind of hideous disfigurement one might expect of the undead. The victims' ears and nose get eaten away. Their lips and gums erode to reveal red, fanglike teeth. Their skin acquires a patchwork of scars, dense pigmentation and deathly pale hues, reflecting underlying anemia. Because anemia can be treated with blood transfusions, some historians speculate that

LIGHT-ACTIVATED DRUGS used in photodynamic therapy could treat diseases of the eye, cancers such as those of the esophagus, and coronary artery disease.

In the dark ages people with porphyria might have tried drinking blood as a folk remedy. Whatever the truth of this claim, those with congenital erythropoietic porphyria would certainly have learned not to venture outside during the day. They might have learned to avoid garlic, too, for some chemicals in garlic are thought to exacerbate the symptoms of the disease porphyria, turning a mild attack into an agonizing reaction.

While struggling to find a cure for porphyria, scientists came to realize that porphyrins could be not just a problem but a tool for medicine. If a porphyrin is injected into diseased tissue, such as a cancerous tumor, it can be activated by light to destroy that tissue. The procedure is known as photodynamic therapy, or PDT, and has grown from an improbable treatment for cancer in the 1970s to a sophisticated and effective weapon against a diverse array of malignancies today and, most recently, for macular degeneration and pathologic myopia, common causes of adult blindness. Ongoing research includes pioneering treatments for coronary artery disease, AIDS, autoimmune diseases, transplantation rejection and leukemia.

Molecular Mechanisms

THE SUBSTANCES at the heart of porphyria and photodynamic therapy are among the oldest and most important of all biological molecules, because they orchestrate the two most critical energy-generating processes of life: photosynthesis and oxygen respiration. Porphyrins make up a large family of closely related compounds, a colorful set of evolutionary variations on a theme. All porphyrins have in common a flat ring (composed of carbon and nitrogen) with a central hole, which provides space for a metal ion such as iron or magnesium to bind to it. When aligned correctly in the grip of the porphyrin rings, these metal atoms catalyze the most fundamental energy-generating processes in biology. Chlorophyll, the plant pigment that absorbs the energy of sunlight in photosynthesis, is a porphyrin, as is heme, which is at the heart of the oxygen-transporter protein hemoglobin and of many enzymes vital for life, including cytochrome oxidase (which generates energy by transferring electrons to oxygen in a critical step of cellular respiration).

Porphyria arises because of a flaw in the body's heme-making machinery. The body produces heme and other porphyrins in a series of eight coordinated stages, each catalyzed by a separate enzyme. Iron is added at the end to make heme. In porphyria, one of the steps does not occur, leading to a backlog of the intermediate compounds produced earlier in the sequence. The body has not evolved to dispose of these intermediates efficiently, so it dumps them, often in the skin. The intermediates do not damage the skin directly, but many of them cause trouble indirectly. Metal-free porphyrins (as well as metalloporphyrins containing metals that do not interact with the porphyrin ring) can become excited when they absorb light at certain wavelengths; their electrons jump into higher-energy orbitals. The molecules can then transmit their excitation to other molecules having the right kind of bonds, especially oxygen, to produce reactive singlet oxygen and other highly reactive and destructive molecules known as free radicals. Metal-free porphyrins, in other words, are not the agents, but rather the brokers, of destruction. They catalyze the production of toxic forms of oxygen.

Photosensitive reactions are not necessarily harmful. Their beneficial effects have been known since ancient times. In particular, some seeds and fruits contain photosensitive chemicals (photosensitizers) called psoralens, which indirectly led scientists to experiment with porphyrins. Psoralens have been used to treat skin conditions in Egypt and India for several thousand years. They were first incorporated into modern medicine by Egyptian dermatologist Abdel Monem El Mofty of Cairo University some 60 years ago, when he began treating patients with vitiligo (a disease that leaves irregular patches of skin without pigment) and, later, those with psoriasis using purified psoralens and sunlight. When activated by light, psoralens react with DNA in proliferating cells to kill them.

Two American dermatologists, the late Aaron B. Lerner of Yale University and the late Thomas B. Fitzpatrick of Harvard University, were struck by the potential of psoralens. In the 1960s they showed that psoralens are activated by ultraviolet (UVA) rays, and the researchers later refined psoralen therapy using an ultraviolet lamp similar to those used in solaria today. Their method became known as PUVA (short for psoralen with UVA) and is now one of the most effective treatments for psoriasis and other skin conditions.

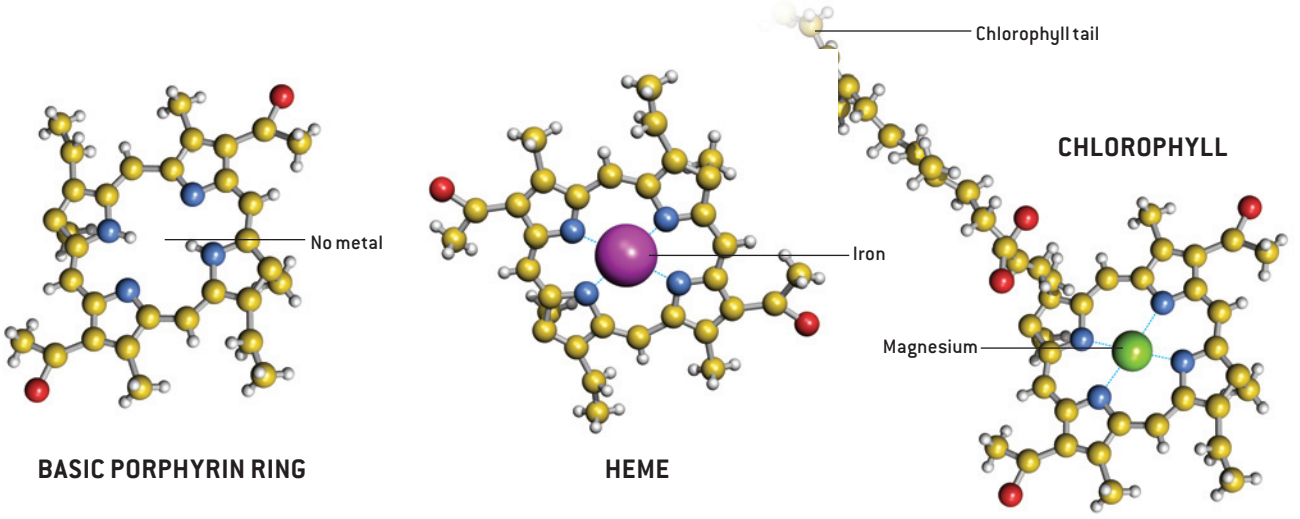
A Way to Kill Cancer Cells?

IN THE EARLY 1970S the success of PUVA impressed Thomas J. Dougherty of the Roswell Park Cancer Institute in Buffalo, N.Y., leading him to wonder if a variant of it could be effective against cancer. Activated psoralens can kill rogue cells to settle inflammation, but in comparison with porphyrins they are not potent photosensitizers. If psoralens could kill individual cells, could porphyrins perhaps devour whole tumors? His idea was the beginning of true photodynamic therapy, in which photosensitizers catalyze the production of

PHOTOGRAPH BY SAM OGDEN (*preceding page*)

Overview/*Light Therapy*

- In photodynamic therapy, light-activated chemicals called porphyrins are used to destroy fast-growing cells and tissue. Doctors could apply the treatment to a variety of ailments, including age-related macular degeneration, tumors and atherosclerotic plaques.
- A few porphyrin drugs are on the market, and several others are undergoing human trials.
- Researchers got the idea for photodynamic therapy from their knowledge of the rare disease porphyria, in which porphyrins accumulate in the skin and certain organs. Unless the disease is managed, victims of the severest type of porphyria can become disfigured, leading some researchers to speculate that they may have inspired medieval vampire legends.



PORPHYRINS all have in common a flat ring, mainly composed of carbon and nitrogen, and a central hole where a metal ion can sit. The basic ring (*far left*) becomes caustic when exposed to light; molecules useful for photodynamic therapy also share this trait. Nontoxic examples include heme (a component of the oxygen transporter hemoglobin) and the chlorophyll that converts light to energy in plants.

oxygen free radicals. The concept was built on pioneering work from German physicians Oscar Raab, then a medical student, and Hermann von Tappeiner, his professor. Around the start of the 20th century, Raab and von Tappeiner showed that acridine, when activated by light, reacts with oxygen to kill protozoa such as paramecia. Von Tappeiner had even gone on to treat patients with skin cancer using eosin, a photosensitive component of coal tar, and white light. Dougherty realized, however, that these early forms of photodynamic therapy lacked the raw power of porphyrins. He also drew on two other medically useful properties of porphyrins discovered in the mid-20th century: porphyrins accumulate selectively in cancer cells and are activated by red light, which penetrates more deeply into biological tissues than do shorter wavelengths, such as white light or UVA.

Dougherty injected a mixture of porphyrins into the bloodstream of mice with mammary tumors. He then waited a couple days for the porphyrins to build up in the tumors before shining red light on them. His early setup was primitive and passed the light from an old slide projector through a 35mm slide colored red. His results were nonetheless spectacular. The light activated the porphyrins within the tumor, which transferred their energy to oxygen in cells to damage the surrounding tissues. In almost every case, the tumors blackened and died after the light treatment. There were no signs of recurrence.

Dougherty and his colleagues published their data in 1975 in the *Journal of the National Cancer Institute,* with the brave title "Photoradiation Therapy II: Cure of Animal Tumors with Hematoporphyrin and Light." Over the next few years they refined their technique by using a low-power laser to focus red light onto the tumors. They went on to treat more than 100 patients in this way, including people with cancers of the breast, lung, prostate and skin. Their outcomes were gratifying, with a "complete or partial response" in 111 of 113 tumors.

Sadly, though, cancer is not so easily beaten. As more physicians started trying their hand with PDT, some serious drawbacks began to emerge. The affinity of porphyrins for tumors turned out to be a bit of an illusion—porphyrins are taken up by any rapidly proliferating tissue, including the skin, leading to photosensitivity. Although Dougherty's original patients were no doubt careful to avoid the sun, nearly 40 percent of them reported burns and skin rashes in the weeks after PDT.

Potency was another issue. The early porphyrin preparations were mixtures, and they were seldom strong enough to kill the entire tumor. Some porphyrins are not efficient at passing energy to oxygen; others are activated only by light that cannot penetrate more than a few millimeters into the tumor. Some biological pigments normally present in tissues, such as hemoglobin and melanin, also absorb light and in doing so can prevent a porphyrin from being activated. Even the porphyrin itself can cause this problem if it accumulates to such high levels that it absorbs all the light in the superficial layers of the tumor, thus preventing penetration into the deeper layers.

Many of these difficulties could not be resolved without the help of specialists from other disciplines. Chemists were needed to create new, synthetic porphyrins, ones that had greater selectivity for tumors and greater potency and that would be activated by wavelengths of light able to reach farther into tissues and tumors. (For each porphyrin, light activation and absorption occur only at particular wavelengths, so the trick is to design a porphyrin that has its absorption maximum at a wavelength that penetrates into biological tissues.) Physicists were needed to design sources that could produce light of particular wavelengths to activate the new porphyrins or that could be attached to fine endoscopes and catheters or even implanted in tissues. Pharmacologists were needed to devise ways of reducing the time that porphyrins spent circulating in the bloodstream, thereby restricting photosensitive side effects. Finally, clinicians were needed to design trials that could

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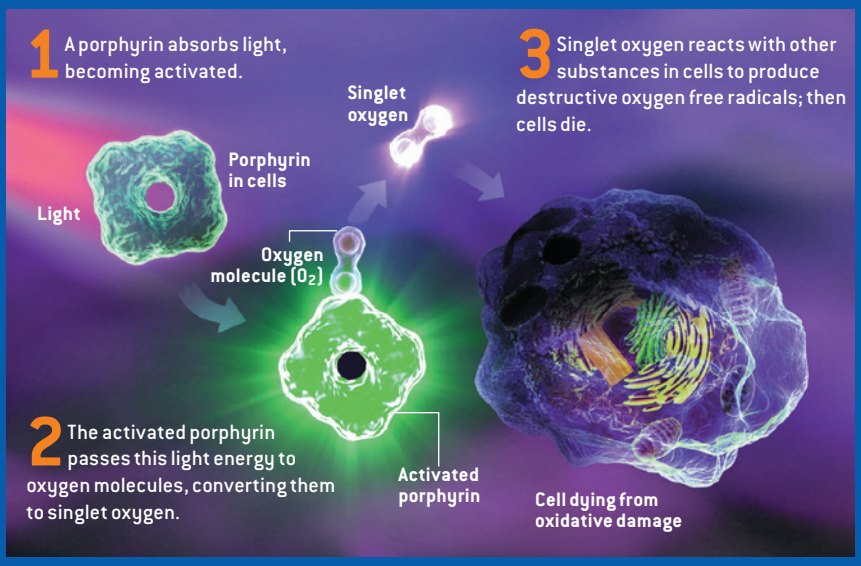
SLIM FILMS

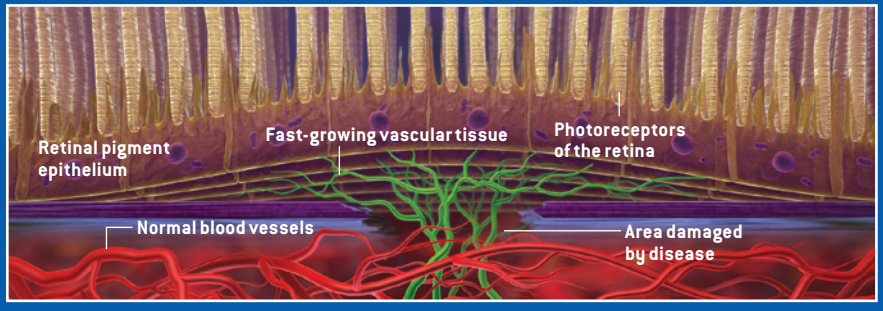
HOW PHOTODYNAMIC THERAPY WORKS

Doctors who administer photodynamic therapy deliver photosensitive chemicals called porphyrins intravenously. These chemicals then collect in rapidly proliferating cells and, when exposed to light, initiate a cascade of molecular reactions that can destroy those cells and the tissues they compose. Some targets for the therapy include abnormal blood vessels in the retinas of people with age-related macular degeneration (the leading cause of adult blindness), cancerous tumors and atherosclerotic plaques in coronary arteries.

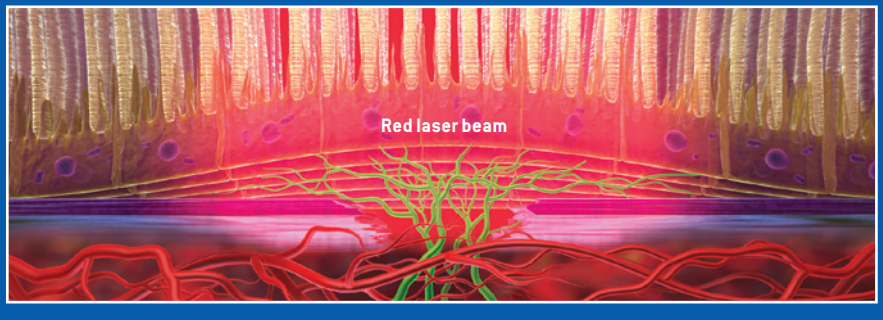
. . . AT THE MOLECULAR LEVEL



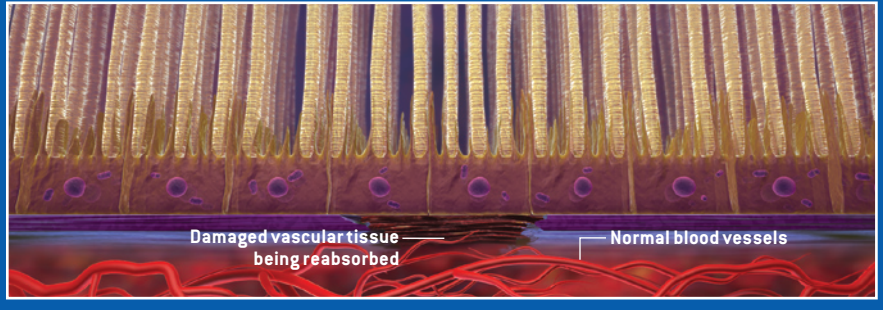
. . . IN THE EYE



1 To treat macular degeneration, a porphyrin (*green*) is injected into a patient's arm. It takes just 15 minutes for the porphyrin to accumulate in abnormal blood vessels under the macula, the central part of the retina responsible for color vision.



2 A red laser light activates the porphyrin, which leads to the destruction of the vascular tissue.

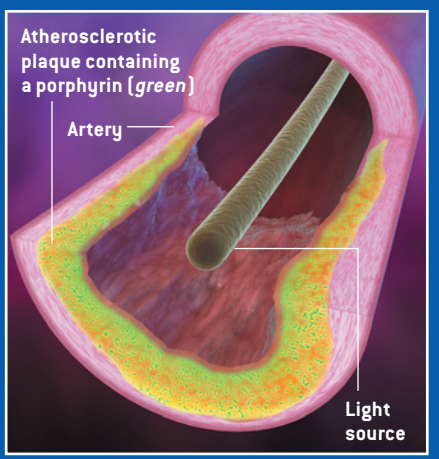


3 After therapy halts damage to the retina, the treated vascular tissue is reabsorbed by the body, and the overlying photoreceptors may settle back into place. Because vessel growth could recur, the patient may require several additional treatments.

... DEEP IN THE BODY

Even long wavelengths of visible light cannot penetrate very far into tissue, so photodynamic therapies for diseased tissue deep within the body require an internal light-delivery system.

1 Here, in an experimental therapy, an optical fiber has been threaded into an artery in which a porphyrin has accumulated in atherosclerotic plaques.



2 The fiber produces red light, activating the porphyrin.

3 Over the course of a few days, the porphyrin destroys unwanted plaques.

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prove an effect and determine the best treatment regimens.

The ideal drug would be not only potent and highly selective for tumors but also broken down quickly into harmless compounds and excreted from the body. The first commercial preparation, porfimer sodium (Photofrin), was approved by the U.S. Food and Drug Administration for the treatment of various cancers. Although it has been helpful against certain cancers (including esophageal, bladder, head and neck, and skin cancers and some stages of lung cancer), it has not been the breakthrough that had been hoped for and cannot yet be considered an integral part of cancer therapy. Surprisingly, though, the first photosensitizing drug to fulfill most of the stringent criteria for potency and efficacy without causing photosensitivity, verteporfin (Visudyne), was approved in April 2000 by the FDA not to treat cancer at all but to prevent blindness. As the theories converged with reality, researchers came to realize that PDT can do far more than destroy tumors.

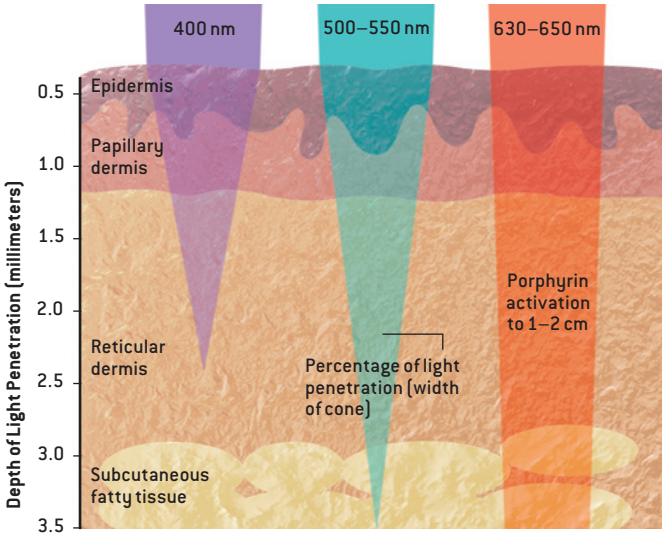
Battling Blindness

ONE THING IT could do, for instance, was combat age-related macular degeneration (AMD), the most common cause of legal blindness in our maturing Western population. Most people who acquire AMD have a benign form and do not lose their sight, but about a tenth have a much more aggressive type called wet AMD. In this case, abnormal, leaking blood vessels, like miniature knots of varicose veins, grow underneath the retina and ultimately damage the sharp central vision required for reading and driving. As the disease progresses, central vision is obliterated, making it impossible to recognize people's faces or the details of objects.

Most attempts to hinder this grimly inexorable process have failed. Dietary antioxidants may be able to delay the onset of the disorder but have little effect on the progression of established disease. Until recently, the only treatment proved to slow the progression of wet AMD was a technique called laser photocoagulation. The procedure involves applying a thermal laser to the blood vessels to fuse them and thus halt their growth. Unfortunately, the laser also burns the normal retina and so destroys a small region to prevent later loss of vision in the rest of the eye. Whether this is worth it depends on the area of the retina that needs to be treated. For most people diagnosed with wet AMD, the area is located below the critical central part of vision or is already too large to benefit from laser coagulation.

Against this depressing backdrop, researchers at Harvard and at the biotechnology firm QLT, Inc., in Vancouver, B.C., reasoned that PDT might halt the growth of these blood vessels and delay or even prevent blindness. If porphyrins could accumulate in any rapidly proliferating tissue—the very problem in cancer—then perhaps they could also accumulate in the blood vessels growing under the retina. Verteporfin, a novel synthetic porphyrin, seemed promising because it had a good track record in preclinical animal studies at QLT and at the University of British Columbia in the late 1980s and early 1990s.

Verteporfin accumulates in abnormal retinal vessels re-



EACH WAVELENGTH OF LIGHT reaches a different depth in tissues, and any given porphyrin absorbs light at specific wavelengths. A porphyrin activated by deeper-penetrating light might be best for treating an internal tumor. In contrast to porphyrins, the psoralens used in PUVA treatments for psoriasis are activated by near-ultraviolet light (400 nanometers), which barely penetrates the skin.

markably quickly: within 15 minutes of injection into an arm vein. When activated by red laser light, verteporfin seals off the vessels, sparing the overlying retina. Any blood vessels that grow back can be nipped in the bud by further treatments. Two major clinical trials, headed by Neil M. Bressler of the Wilmer Eye Institute at Johns Hopkins University, confirmed that PDT can be given six or seven times over a three-year period without damaging a healthy retina. For people with the most aggressive form of AMD (with mostly "classic" lesions), verteporfin halved the risk of moderate or serious vision loss over a two-year period. The effect is sustained over at least three or four years: patients who are not treated lose as much vision in three months as those treated with verteporfin lose in three years. The treatment also worked, though not as well, for people with less aggressive types of AMD and for those with related diseases such as pathologic myopia and ocular histoplasmosis syndrome. Only a small proportion of patients suffered from sunburns or other adverse reactions, rarely more than 24 hours after the procedure was done.

Some participants in the trials gained little benefit from PDT. For many of them, the disease may have already progressed too far. A reanalysis of clinical data presented by Bressler in April 2002 at the International Congress of Ophthalmology in Sydney showed that smaller lesions respond much better to treatment than older, larger ones, implying that early detection and treatment may optimize the benefits of PDT.

Other Treatment Avenues

THE SUCCESS OF ophthalmic PDT has inspired research activity in other fields but also reveals the drawbacks of the treatment. In particular, even red light penetrates no more than a few centimeters into biological tissues [*see illustration above*]. This limitation threatens the utility of PDT in internal medicine—its significance might seem to be skin deep. There are ways of turning PDT inward, however. One ingenious idea is called photoangioplasty, which is now being used to treat coronary artery disease.

Coronary angioplasty is a minimally invasive procedure for treating arteries affected by atherosclerosis. It uses a tiny balloon to open arteries, so that atherosclerotic plaques do not occlude the entire vessel. Photoangioplasty could sidestep many of the problems of conventional angioplasty, notably the restenosis (renarrowing) of treated arteries. The procedure involves injecting a porphyrin into the bloodstream, waiting for it to build up in the damaged arterial walls and then illuminating the artery from the inside, using a tiny light source attached to the end of a catheter. The light activates the porphyrins in the plaques, destroying the abnormal tissues while sparing the normal walls of the artery. The results of a small human trial testing the safety of the synthetic porphyrin motexafin lutetium were presented in 2002 by Jeffrey J. Popma, then at Brigham and Women's Hospital, at the annual meeting of the American College of Cardiology. Although it is still early in the testing process, the findings fuel hopes for the future: the procedure was safe, and its success at preventing restenosis increased as the dosage increased.

An even more ingenious method for treating deep cancers is the use of self-lighting nanoparticles. Wei Chen of the University of Texas at Arlington recently developed a method of attaching porphyrins to "scintillation luminescence nanoparticles." The nanoparticles generate visible light when exposed to x-rays during radiotherapy, and this in turn activates the porphyrin. The method effectively combines radiotherapy with PDT, optimizing both methods and allowing the treatment of large or deeply buried tumors with relatively low-dose radiotherapy. But how will the bulky nanoparticles be targeted at tumor cells? In January Chen and his colleagues reported preliminary findings suggesting that the whole complex can be attached to folic acid, which is then taken up by folate receptors on cancer cells.

An alternative approach for targeting porphyrins or even nanoparticle complexes at tumors involves attaching them to antibody fragments that recognize cancer cells. Mahendra Deonarain and his colleagues at Imperial College London, who are pioneers of this method, have managed to attach more than 10 porphyrin molecules to a single antibody fragment without destroying its ability to target cancer cells. The group used antibody fragments rather than whole antibodies to reduce the size of the complex and enable it to be cleared from the body more quickly. The team's initial findings, reported in March, showed the complexes accumulating in tumors at 10 times their concentration in blood and 50 times their concentration in muscle.

Accumulation of porphyrins in active and proliferating cells raises the possibility of treating other conditions in which abnormal cell activation or proliferation plays a role—among

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Photodynamic Therapies

The light-activated drugs listed below are a sampling of those on the market or in development.

DRUG	TARGET	MAKER	STATUS
Levulan (5-aminolevulinic acid)	Acne and actinic keratosis (a precancerous skin disorder), Barrett's esophagus (a precancerous condition)	DUSA PHARMACEUTICALS Toronto	On the market for actinic keratosis; phase II trials (relatively small studies in humans) have begun for acne
PHOTOFRIN (porfimer sodium)	Cancers of the esophagus and lung, high-grade dysplasia from Barrett's esophagus	AXCAN SCANDIPHARM Birmingham, Ala.	On the market for esophageal cancer and nonsmall cell lung cancer; FDA-approved for high-grade dysplasia
VISUDYNE (verteporfin)	Age-related macular degeneration, pathologic myopia and ocular histoplasmosis (eye disorders)	QLT, INC., and NOVARTIS OPHTHALMICS Vancouver, B.C., and Duluth, Ga.	On the market
METVIX (methylaminolevulinic acid)	Actinic keratosis, basal cell skin cancer and squamous cell skin cancer	PHOTOCURE Oslo, Norway	FDA-approved for actinic keratosis and basal cell skin cancer
LS11 (talaporfin sodium)	Colorectal cancer, hepatocellular carcinoma, and glioma	LIGHT SCIENCES ONCOLOGY, INC. T Bellevue, Wash.	In phase III trials for colorectal cancer with recurrent liver metastases and for hepatocellular carcinoma; phase II trial for glioma completed in February
Tookad (vascular-targeted PDT)	Prostate cancer	STEBA BIOTECH, France	In phase II/III trials for prostate cancer after radiation therapy

them, infectious diseases. Attempts to treat infections with the pigments had long been frustrated by a limited effect on gram-negative bacteria, which have a complex cell wall that obstructs the uptake of porphyrins into these organisms. One solution, developed by Michael R. Hamblin and his colleagues at Harvard, involved attaching a polymer—usually polylysine, a repetitive chain of the amino acid lysine—to the porphyrin. The polymer disrupts the lipid structure of the bacterial cell wall, enabling the porphyrins to gain entry to the cell. Once inside, they can be activated by light to kill the bacteria. In studies of animals with oral infections and infected wounds, the altered porphyrin showed potent antimicrobial activity against a broad spectrum of gram-negative and gram-positive bacteria. As antibiotic resistance becomes more intractable, targeted antimicrobial PDT could become a useful weapon in the medical arsenal.

Several other, related photodynamic methods hinge on the finding that activated immune cells take up greater amounts of photosensitizing drugs than do quiescent immune cells and red blood cells, sparing the quiet cells from irreversible damage. In most infections, nobody would wish to destroy activated immune cells: they are, after all, responsible for the body's riposte to the infection. In these cases, targeting immune cells would be equivalent to "friendly fire" and would give the infection free rein to pillage the body.

In AIDS, however, the reverse is true. The AIDS virus, HIV, infects the immune cells themselves. Targeting infected immune cells would then be more like eliminating double agents. In the laboratory, HIV-infected immune cells take up porphyrins, thereby becoming vulnerable to light treatment. In patients, the light could be applied either by withdrawing blood, illuminating it and transfusing it back into the body (extracorporeal phototherapy) or by shining red light onto the skin, in what is called transdermal phototherapy. In the transdermal approach, light would eliminate activated immune cells in the circulation as they passed through the skin. Whether the technique will be potent enough to eliminate diseased immune cells in HIV-infected patients remains an open question.

Autoimmune diseases, rejection of organ transplants, and leukemias are also all linked by the common thread of activated and proliferating immune cells. In autoimmune diseases, components of our own body erroneously activate immune cells. These activated clones then proliferate in an effort to destroy the perceived threat—say, the myelin sheath in multiple sclerosis or the collagen in rheumatoid arthritis. When

MORE TO EXPLORE

The Colours of Life: An Introduction to the Chemistry of Porphyrins and Related Compounds. L. R. Milgrom. Oxford University Press, 1997.

Lethal Weapon. P. Moore in *New Scientist,* Vol. 158, No. 2130, pages 40–43; April 18, 1998.

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Targeted Photodynamic Therapy with Multiply-Loaded Recombinant Antibody Fragments. M. Bhatti, G. Yahioglu, L. R. Milgrom, M. Garcia-Maya, K. A. Chester and M. P. Deonarain in *International Journal of Cancer,* Vol. 122, No. 5, pages 1155–1163; March 2008.

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organs are implanted, activated immune cells may multiply to reject the foreign tissue—the transplanted organ or even the body tissues of the new host, in the case of bone marrow transplants. In leukemia, immune cells and their precursors in the bone marrow produce large numbers of nonfunctional cells. In each instance, PDT could potentially eliminate the unwanted immune cells, while preserving the quiescent cells, to maintain a normal immune response to infection. As in HIV infection, the procedure might work either extracorporeally or transdermally. Much of this research is in late-stage preclinical or early clinical trials. For all the cleverness in exploring possible medical applications, though, we can only hope that more extensive clinical studies will bear fruit.