Angiogenesis Inhibitors Link Many Diseases

A new class of compounds that block blood vessel proliferation may provide therapy for diseases as diverse as cancer and diabetic retinopathy

A malignant tumor differs from normal organs in many respects, but one newly recognized difference may open the door to a unique new therapy. The blood supply of an organ, once established, is very stable, but that of a tumor is much more transient. It has long been known that a tumor must recruit new blood vessels in order to obtain nutrients for growth. New results, however, indicate that a tumor must also continually recruit new blood vessels simply to keep from withering away.

If this process of blood vessel recruitment can be interfered with by specific inhibitors, the tumor should shrink. Such agents might also prove useful in other diseases involving abnormal proliferation of blood vessels, including diabetic retinopathy and retrolental fibroplasia, as well as processes involving resorption of bone.

The process of recruiting new blood vessels is known as angiogenesis. In the early 1970's, Judah Folkman and his colleagues at Children's Hospital Medical Center in Boston discovered that tumors secrete a substance, known as tumor angiogenesis factor or TAF, that induces existing blood vessels to grow toward and to infiltrate the tumor, providing it with the nutrients necessary for growth. This process is readily seen in the most common animal model, the implantation of a few tumor cells in the cornea of rabbit eyes. Within 7 days, blood vessels can be clearly seen infiltrating the tumor cells. Tumor angiogenesis factors have subsequently also been isolated by Shant Kumar of the Patterson Laboratories in Manchester, England, and Alan Fenselau of the Johns Hopkins University School of Medicine.

Tumors are not the only source of angiogenic materials. At the end of last year, Bert M. Glaser and Patricia A. D'Amore of Johns Hopkins reported that they had obtained an angiogenic substance from the retina of several species. More recently Chung-Ho Chen of Johns Hopkins has obtained another from the vitreous humor of fetal calf eyes. Dennis Gosporodowicz of the University of California at San Francisco appears to have isolated an angiogenic substance from corpus luteum. Glaser and D'Amore have also isolated chemotactic agents—chemicals that stimulate endothelial cells to migrate toward them—from bovine retina; these chemotactic chemicals may be related to angiogenesis factors.

The angiogenesis factors are present in such small quantities that they have not yet been purified and characterized. Little is known about their mechanism of action either, except on a gross anatomical scale. Electron microscope studies by Dianne Ausprunk in Folkman's laboratory have shown that blood vessel growth in response to tumors occurs in two distinct phases. In the first stage, endothelial cells at the tip of the affected vessels become elongated and form a lumen as they attempt to migrate toward the tumor; during this stage, the cells also release proteolytic and collagenolytic enzymes that degrade the intercellular matrix through which they must migrate. In the second stage, the cells immediately behind those at the tip proliferate, pushing the lumen forward. Interference with any of these steps should block new vascular growth.

It is not necessary, of course, to know precisely what TAF is to try to block its activity. The first steps toward identification of such an inhibitor were taken in 1973 by Reuben Eisenstein, Klaus E. Kuettner, and Nino Sorgente of the Rush–Presbyterian–St. Luke's Medical Center in Chicago. They reasoned that cartilage, which is not normally vascularized, might contain an angiogenesis inhibitor (AI, also known as an antiinvasion factor or AIF). They cut cartilage into small pieces and implanted them on the chorioallantoic membrane of chick embryos. They observed that the cartilage was not invaded by vascular mesenchymal cells (a process analogous to the proliferation of blood vessels) or by blood vessels, whereas other implanted tissues were invaded by both. When the cartilage was extracted with a guanidine solution, however, the mesenchymal tissue and blood vessels did invade it. Subsequently, Folkman and his colleagues implanted pieces of cartilage in rabbit corneas between the vascular system and injected tumor cells and observed that the tumor did not become vascularized.

Somewhat later, Robert S. Langer of the Massachusetts Institute of Technology and Folkman injected a partially purified guanidine extract of cartilage into rabbit corneas and again observed inhibition of tumor vascularization. This assay proved difficult, however, because the AI diffuses away from the injection site rather rapidly. They thus spent more than 2 years developing controlled-release polymers that would serve as a depot for chemicals of high molecular weight (the cartilage AI is believed to be a protein with a mass between 12,000 and 28,000 daltons). This proved to be a productive area of research on its own and has opened up the possibility of sustained-release depots for insulin, heparin, interferon, and a variety of other biological materials.

Langer implanted polymer depots containing the partially purified guanidine extract in rabbit corneas and obtained the same results as he had with cartilage. These polymers are now used routinely by Langer and other investigators in studies of the inhibitors, particularly in the bioassays used during inhibitor purification. But before they spent a lot of time isolating the cartilage AI, Langer says, "we wanted to feel that it was an important substance"; in particular, they wanted to know that it would work when administered through the bloodstream. Because of the small amount of cartilage AI available, it was necessary to introduce the agent into the right carotid artery—which leads directly to the right eye—in rabbits by slow infusion. After passing through the right eye, the inhibitor would pass through the bloodstream and be greatly diluted before reaching the left eye, so that the latter could serve as a control.

When tumor cells were implanted in both corneas, Langer and Folkman recently reported, new blood vessels in the control animals and in the left eyes grew 32 to 35 times as fast as vessels in the right eyes. The number of capillaries in the left eyes also increased more than 25-fold during the 6-day infusion period, whereas there was little or no increase in the number of capillaries in the right eyes. In a second set of experiments to determine what would happen if the tu-

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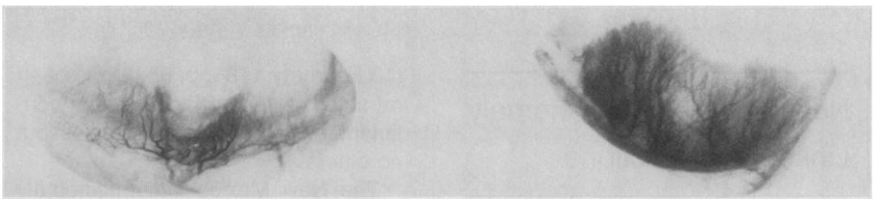
mor were in physical contact with blood vessels, a different type of tumor was implanted directly onto the dense capillary bed of the conjunctiva of mouse eyes, then excised 7 days later and weighed. Tumors from the control animals and from the left eyes of infused animals weighed an average of 41 times more than those from the right eyes. These experiments together show that infusion of AI's into the bloodstream is effective, even when the tumor is in direct contact with host blood vessels.

More recently, Eisenstein, now at the Mt. Sinai Medical Center in Milwaukee, has reported "statistically significant but not biologically significant" regression of mammary tumors in mice injected with an AI isolated from aorta and a more substantial regression of a fibrosarcoma. The inhibitor was only partially purified, was available only in very limited quantities, and was not infused continuously, so it is not surprising that its effects were modest. But this experiment also showed that systemic injection can be effective.

Angiogenesis inhibition is by now a well-documented and fairly common phenomenon. Langer, Kuettner, and Sorgente, who is now at the University of Southern California School of Medicine, have each isolated an AI from cartilage. Langer and Sorgente use veal scapulas as a source because they are available in large quantities, but Kuettner uses bovine nasal septums because they can be obtained fresher. Eisenstein has isolated the previously mentioned inhibitor from aortas, which are also avascular. Arnall Patz, Gerard A. Lutty, and Robert J. Mello of the Johns Hopkins School of Medicine have obtained an AI from vitreous humor; it appears to be present in adults of all species tested so far, but they use cow eyes as a source. Several groups are studying naturally occurring protease and collagenase inhibitors that could block the degradation of the intercellular matrix and thereby prevent vascularization. Among them are Daniel Rifkin of the New York University School of Medicine, Alan Barrett and John Reynolds of the Strangeways Laboratories in Cambridge, England, Victor Hatcher of the Albert Einstein College of Medicine, and Kuettner.

It seems likely that there are actually several different inhibitors and all seem to be positively charged proteins. Even the three groups working with materials isolated from cartilage may not be studying the same molecule. Langer and Sorgente think that theirs is a single protein with a mass that Sorgente says is between 20,000 and 25,000 daltons; Kuettner says that he has isolated at least five separate but related proteins that block all three steps involved in proliferation. Langer's cartilage AI also inhibits the enzyme trypsin; Sorgente's does not. Sorgente and Kathleen C. Dorey have shown that AI inhibits the enzyme ornithine decarboxylase, which suggests that it interferes with the early part of the cell cycle. Eisenstein's aortic AI, meanwhile, appears to have a mass between 3,500 and 10,000 daltons. It also inhibits proteases and collagenase, activities that Eisenstein says are distinct from endothelial cell inhibition.



Inhibitor blocks vessel growth

Cartilage AI in polymer implant (left) blocks vessel growth toward tumor in cornea; in absence of inhibitor (right), vessels grow freely. [Source: Robert Langer, MIT]

Purification and characterization of the inhibitors has, in fact, been the major bottleneck in angiogenesis studies. Each purification step requires a bioassay of the substance. These generally involve inhibition of vessel growth in rabbit eyes, inhibition of the invasion of mesenchymal tissues in the chick chorioallantoic membrane, or inhibition of the growth of endothelial cells in culture. Each of these techniques has limitations, and each requires significant amounts of the inhibitor. "The person who develops a simpler assay," says Folkman, "will open the field wide."

One new candidate assay has been developed recently by Kuettner and Bendicht U. Pauli of Rush–Presbyterian–St. Luke's. They use a small stainless steel cube in which one side has been replaced by extracted cartilage. The device is placed in a culture dish, and tumor cells are implanted on holes on the face opposite the cartilage. The cells would normally invade the cartilage, Kuettner says, and inhibition of this invasion can be measured accurately.

When the field is "opened wide," there are many potential uses for AI's. The most obvious of these is as an anticancer agent. For many years, most investigators assumed that angiogenesis inhibitors would be useful only for metastases, since established tumors already have a blood supply. In 1978, though, Ausprunk and Folkman demonstrated that tumor-induced blood vessels regress and die when TAF is removed. But, says Langer, "we have never had enough of the cartilage material to test it on an established tumor." "We have always assumed," adds Folkman, "that the cartilage AI is simply a prototype, and that more potent inhibitors will be discovered."

Angiogenesis inhibitors would have many advantages in cancer therapy. Since they are naturally occurring substances and highly specific for endothelial cells, they should have few, if any, side effects. Moreover, the inhibitors have no direct effect on tumor cells—cultured tumor cells grow normally in the presence of high concentrations of any of the AI's—so there may be less possibility of resistance developing. "My guess," says Folkman, "is that the inhibitors and chemotherapeutic agents should be highly synergistic."

Other possibilities are somewhat more speculative, but nonetheless interesting. The discovery in Patz's lab of naturally occurring AI's and angiogenesis substances in the eye suggest potential mechanisms for diabetic retinopathy, sickle cell retinopathy, and retrolental fibroplasia. The first two are disorders accompanying diabetes or sickle cell disease in which blood vessels proliferate in the eyes, eventually producing blindness. The last is found in newborn infants exposed to high concentrations of oxygen; the oxygen closes blood vessels in the eyes and, after its use is halted, new vessels proliferate, causing blindness. It seems possible that all three disorders involve some disruption of the function of angiogenesis factors or inhibitors, and that exogenous inhibitor might eventually be useful for preventive therapy.

Kuettner and John Horton of the Harvard School of Dentistry have also found that the cartilage AI inhibits the proliferation and invasion of osteoclasts, the cells that are responsible for resorption (dissolution) of bone. Resorption is a problem both in arthritis, where joints are attacked and dissolved, and in bone transplants, since foreign bone tissue is often eventually resorbed. A specific inhibitor of osteoclasts might thus be useful in treatment of both disorders.

—THOMAS H. MAUGH II