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REVIEW

Angiogenesis Inhibitors and Their Delivery Systems

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Abstract

The extensive vascularity of solid tumors has been recognized for over 100 yr (*1*). However, only in the past 15 yr has the importance of this phenomenon been appreciated and only in the past 6 yr has the possibility of chemical interference been apparent. In this article, we review the emerging field of tumor vascularization inhibitors.

Index Entries: Angiogenesis inhibitors, delivery systems of; inhibitors, angiogenesis, and their delivery systems; controlled release systems, for angiogenesis inhibitors.

I. Eary Studies

Although Virchow, in the nineteenth century, recognized that tumors are richly supplied by blood vessels (*1*), Algire and Chalkey first appreciated that tumors continuously elicit new blood vessel growth from the host (*2*). This process is called tumor angiogenesis. They also suggested that this ability might permit autonomous tumor growth. Despite this observation little progress was made in the next 20 yr, principally because of the lack of an experimental system with which to study angiogenesis. In the 1960s, Folkman and his colleagues were exploring the use of isolated perfused organs to study the early phases of tumor growth. They noted that all tumor implants failed to vascularize in these systems and stopped growing at a diameter of less than 1 mm. However, the tumors remained alive and

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when they were reimplanted into normal animals, they became vascularized and grew rapidly. The reason for these results was that the perfusion procedure caused damage to the blood vessels in the isolated organ (*3*).

Two additional lines of evidence suggested that if it were possible to inhibit angiogenesis, a tumor nodule might remain dormant at a few mm in diameter: (1) Tumor implants in the anterior chamber of the rabbit eye remained avascular because new vessels could not reach them through the aqueous humor. These tumors never grew beyond 1 mm in diameter (*4*). (2) Tumor spheroids grown in soft agar medium that was continuously changed also reached a steady state of growth where generation of new cells and death of old cells became balanced. These spheroids ranged from 1 to 4 mm, each containing less than 1 million cells (*5*). These studies suggested that antiangiogenesis might someday become a potential therapeutic approach to controlling tumor growth (*6*).

Simultaneously, several studies pointed to the possibility that tumor neovascularization is chemically mediated. In 1968, Shubik and Greenblat placed a tumor inside a chamber containing a 0.45 μm Millipore filter. A tumor on one side of the filter induced new blood vessel growth on the other side. Since the tumor cells could not cross the filter, vascularization must have been induced by a diffusible material (*7*). Subsequently, Folkman and coworkers found that a soluble extract from tumor cells also elicited neovascularization when injected into rats (*8*).

The possibility that mediators of antiangiogenesis exist was suggested by studies by Eisenstein et al. They implanted different tissues on the highly vascular chick chorioallantoic membrane (CAM). Of these, only cartilage failed to become vascularized (*9*). To test the ability of cartilage to inhibit tumor angiogenesis, Brem and Folkman implanted slices of neonatal rabbit cartilage adjacent to pieces of V2 carcinoma in the rabbit cornea. A highly significant inhibition of neovascularization was observed (*10*). Sorgente and coworkers reported that cartilage contained protease inhibitory activity that could be extracted with 1*M* guanidine. When the extracted cartilage was implanted on the CAM it became vascularized (*11*). These findings, coupled with the observation that embryonic cartilage is vascularized, but loses its blood vessels during the early neonatal period (*12*), provided the impetus for us to explore neonatal cartilage as a source of an angiogenesis inhibitor. Scapular cartilage from newborn calves (veal) was selected since it is plentiful and easy to obtain. The initial phases of our work focused on two areas: (1) the development of effective delivery systems for the inhibitor, and (2) the extraction and partial purification of the inhibitor.

II. Delivery Systems

The bioassay for studying the inhibitor consists of placing a piece of tumor in the cornea of a rabbit and monitoring the growth of new vessels from the corneal edge (limbus) toward the tumor. We wanted to deliver inhibitor to the tumor to see it if decreased the rate of blood vessel growth. Because there are normally no blood vessels in the cornea, this assay provides a quantitative measure of angiogenesis

inhibition. No other assay site has the advantage of no pre-existing blood vessels. This assay takes 30 d and the purified fractions of the cartilage material are highly soluble, so that once they were added to the cornea they disappeared quickly. Therefore, we needed a small sustained—release system to provide steady diffusion into the region near the tumor. Such a system had to be noninflammatory and capable of continuously releasing molecules as large as proteins and polysaccharides present in cartilage. The only biocompatible polymer systems capable of sustained release were limited by the size (mw <600) of the molecules that could diffuse through the polymers. Therefore, we looked for new polymers and new ways of placing drugs in these polymers. We found that by dissolving the polymer in an appropriate solvent and adding the macromolecule in powder form, a mixture was obtained which, when cast in a mold, dried, and placed in water, was able to release incorporated macromolecules. We tested a number of polymer systems for tissue biocompatibility and release kinetics. Only poly-2-hydroxylethylmethacrylate (Hydron®) did not cause inflammation in the rabbit cornea. Other polymers such as ethylene–vinyl acetate copolymer could be rendered noninflammatory by washing with absolute alcohol, which extracts inflammatory impurities such as antioxidants (*13*). Our best long-term release results were obtained with ethylene–vinyl acetate copolymer, using methylene chloride as a casting solvent. Release periods of a few days to a week were also obtained using Hydron® or polyvinylalcohol. Figure 1 shows the results of an early study in which four different proteins ranging in molecular weight from 14,400 (lysozyme) to 250,000 (catalase) were released continuously from small (2-mm diameter, 1.5-mm deep), cone-shaped, ethylene–vinyl acetate copolymer pellets for over 100 d. Over 80% of the escaping enzyme was biochemically active. In other tests,

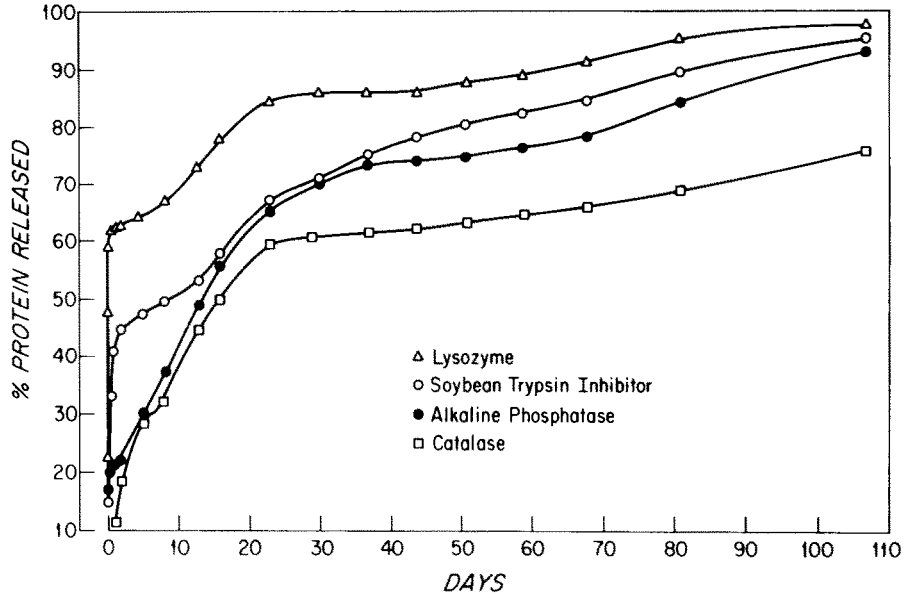

Fig. 1. Release of four different proteins from ethylene–vinyl acetate copolymer pellets (*14*).

larger molecules (mw 2 million), such as polysaccharides and polynucleotides, were also released for long time periods (*14*).

The incorporation of powdered macromolecules during polymer matrix casting creates a series of interconnecting pores through which dissolved drug can then diffuse (Fig. 2). Release rates can be changed over several thousandfold by altering factors such as drug powder particle size and the number of particles placed in the polymers (*15*). Subsequent studies have led to the development of devices with highly reproducible release rates (*16*), have demonstrated that in vitro and in vivo release kinetics of any individual macromolecule from these systems are identical (*17*), have developed specially shaped systems that release the macromolecule at a constant rate (*18*), and created systems in which release rates can be externally controlled using magnetism (*19*). A recent development is the use of inert carrier molecules, such as albumin (used to create the porous network), that can be placed inside the polymer along with microgram quantities of angiogenesis inhibitor, thereby permitting smaller quantities of inhibitors to be tested (*20*).

These ethylene–vinyl acetate copolymer systems have been widely used in bioassays for angiogenesis stimulators and inhibitors (*21–26*) and hold promise for the delivery of insulin (*27*), vaccines (*28*), heparin (*29*), and possibly other compounds.

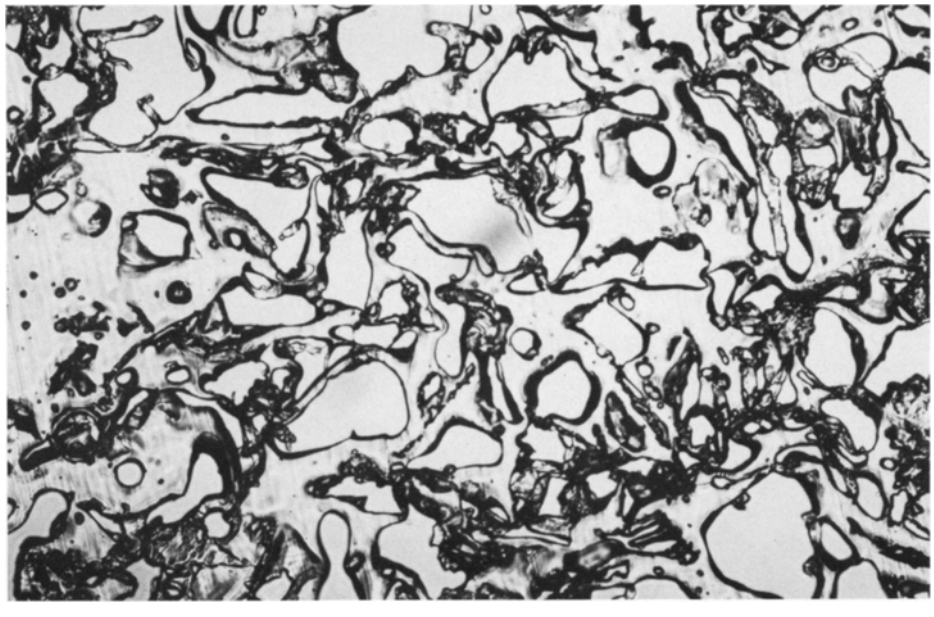

Fig. 2. A 5-μm cross section of an ethylene–vinyl acetate copolymer pellet originally containing albumin that had been released for 5 months; the pores are left behind where the albumin was originally.

III. Isolation of an Angiogenesis Inhibitor

Cartilage was excised from fresh veal scapula and the connective tissue was removed. Extracts of cartilage were then made by a variety of methods, including simple aqueous extraction, guanidine extraction, and Ringer's extraction, and were further purified using $(NH_4)_2SO_4$ precipitation and column chromatography. Most of the extracts caused severe inflammation. Only one fraction had angiogenesis-inhibiting activity. This fraction was obtained by extracting with 1 *M* guanidine, followed by trypsin-affinity chromatography. All extracts were dialyzed exhaustively against water at 4°C, passed through 0.45-μm Millipore filters, and lyophilized. Sterile technique was followed thereafter (*30*).

To test fractions for angiogenesis inhibition, pieces of V2 carcinoma were implanted adjacent to slow release polymers containing test fractions in the rabbit cornea (Fig. 3a). The rate of vessel growth (as determined by the length of the longest vessel) toward the tumor was measured.

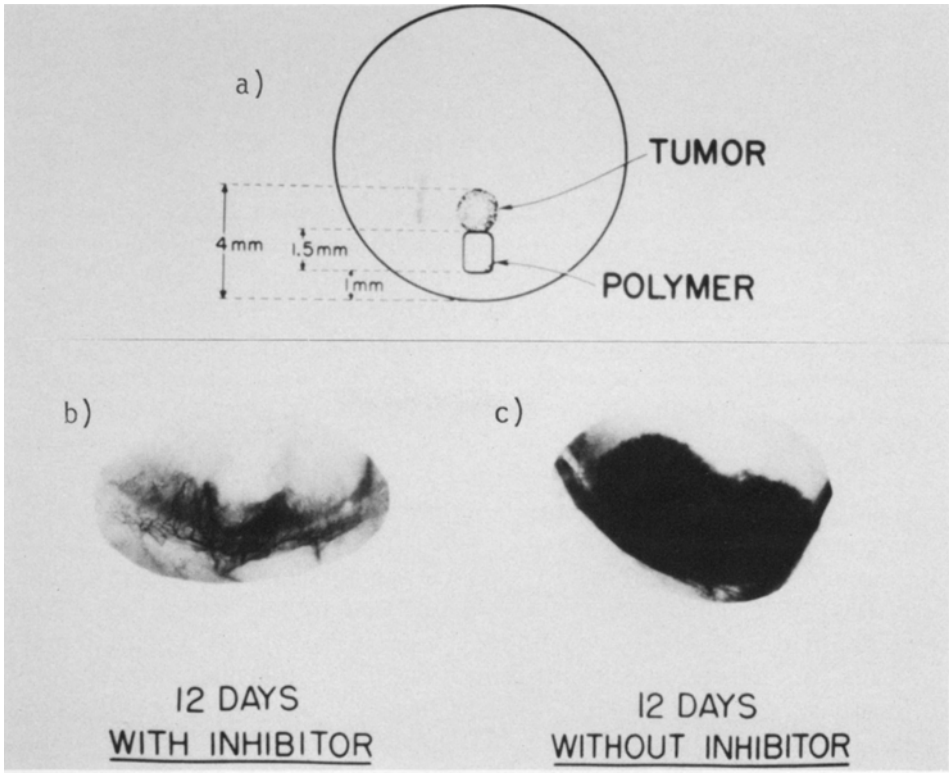

Fig. 3. (a) Rabbit cornea with tumor and polymer; (b) in presence of inhibitor, vessels are sparse and fail to grow in zone around polymer. Tumor is avascular. (c) In absence of inhibitor (empty polymer), vessels appear as dense carpet sweeping over the polymer. The tumor has vascularized and is growing rapidly (*30*).

More than 20 cartilage fractions were tested in over 500 corneas. The active fraction, a group of proteins possessing strong trypsin inhibitory activity, isolated by affinity chromatography, caused a 70% reduction in the vascular growth rate. No other fraction caused any significant reduction. In several sets of experiments consisting of 21 corneas with the active fraction and 16 controls, all control rabbits had to be sacrificed by 3 weeks because their vascularized tumors were so large. In contrast, 10 of the 21 tumors in the experimental group failed to vascularize during a three-month period, the time course of the experiment. Many of the vessels in the experimental group actually regressed (*30*).

In addition, there were striking qualitative differences in corneas receiving the inhibitor compared to control corneas. When the polymers were empty or contained an inactive test fraction, vessels appeared as a dense front sweeping over the polymer toward the tumor (Fig. 3c). In contrast, when the polymer contained the active inhibitor, vessels were sparse, grew slowly and failed to grow in a zone surrounding the polymer (Fig. 3b).

IV. Continuous Infusion of the Cartilage-Derived Angiogenesis Inhibitor

We wanted next to determine if the inhibitor would be effective when administered parenterally since this more closely approximates the clinical situation. Studies in rabbits and mice were conducted. In the rabbit experiments, an indwelling catheter was placed in the right common carotid artery (*31*) so that Ringer's solution containing inhibitor could be infused. Placement of the infusion catheter in the right common carotid artery caused each rabbit to receive a high dose in the right eye and a low dose in the left eye, as proven by 199Tc scanning (*32*) (Fig. 4). V2 carcinomas were implanted (1.5-mm pieces) in the rabbit cornea 1.0 mm from the corneal edge. Rabbit corneas were examined daily with a Zeiss slit-lamp stereoscope.

Three sets of infusion experiments were conducted with similar results (Fig. 5). Three experimental rabbits were infused with the inhibitor. Twenty-one controls were infused with lactated Ringer's and four controls were also infused with trasylol (bovine trypsin inhibitor). Average corneal vessel growth rate for the Ringer's control was 0.32 mm/d and for the trasylol control it was 0.46 mm/d over the 6-d infusion period. In contrast, the average growth rate of vessels in the treated eyes was 0.01 mm/d (*32*). Blood vessel growth rate was the same as that of control eyes before the infusion of inhibitor began. Growth of vessels ceased during the infusion, and then returned to the growth rate of controls after the inhibitor was discontinued (although in some instances there was a lag phase in vessel growth following cessation of inhibitor). In the animals receiving inhibitor, the contralateral eye was exposed to a very low concentration of the inhibitor. The contralateral eye, therefore, served as an additional internal control. Vascular growth in the contralateral eye of animals receiving the inhibitor was the same as vascular growth in animals infused with Ringer's or trasylol (Fig. 5).

The angiogenesis inhibitor affected the density of vessels, as well as their rate of growth. Although all control corneas showed an increase in vessel number from

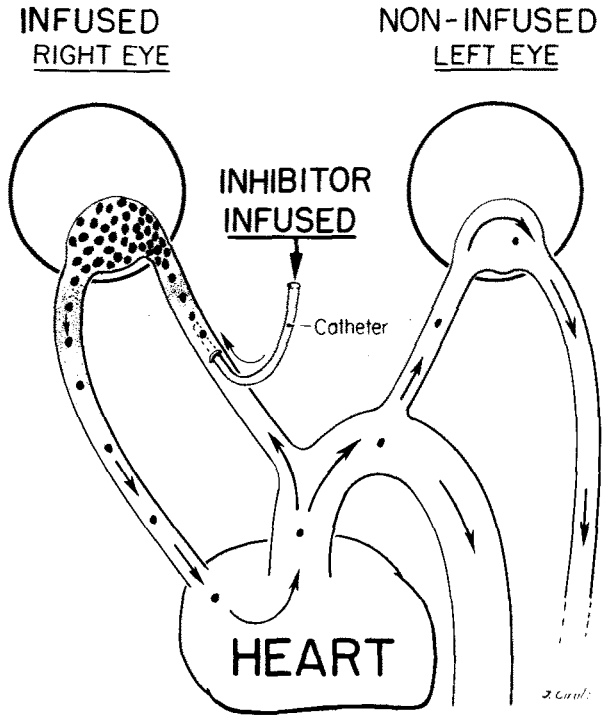

Fig. 4. Catheter in right carotid artery permits a high concentration of inhibitor into the right eye, and a low concentration into the left eye (*32*).

approximately four to over 100 vessels per cornea during the 6-d infusion period, two of the three treated corneas showed no increase in vessel density. The third cornea showed only a small increse in vessel density and a 0.1-mm increase in length. Yet even this cornea was markedly less vascularized than the most mildly vascularized corneas of the controls (*32*).

All 28 controls developed large bulging tumors. In contrast, tumors in the treated eyes grew much more slowly. In two of the three cases, the tumors eventually regressed.

In the rabbit experiments the tumor was separated from the host's vascular bed, and this permitted the daily measurement of vessel growth rate. However, clinically, most tumors are situated or arise in a previously existing vascular bed; thus tumor and host vasculature are contiguous. Therefore, we did a second set of experiments in which the tumor implant was placed next to a vascular bed. Mouse melanoma (B16) was implanted directly in the dense capillary bed of the mouse conjunctiva.

In these experiments, the inhibitor was infused [using the carotid infusion system of Brown and Goffinet (*33*)], into 90 mice with bilateral subconjunctival implants of B16 melanoma. Control animals received Ringer's solution, bovine serum albumin, or trasylol. Four milligrams of the inhibitor or control proteins were infused per day; 7 d after the infusion began, all animals were sacrificed and the tumors were excised. The control tumors weighed 7.8 ± 3.9 mg, whereas the

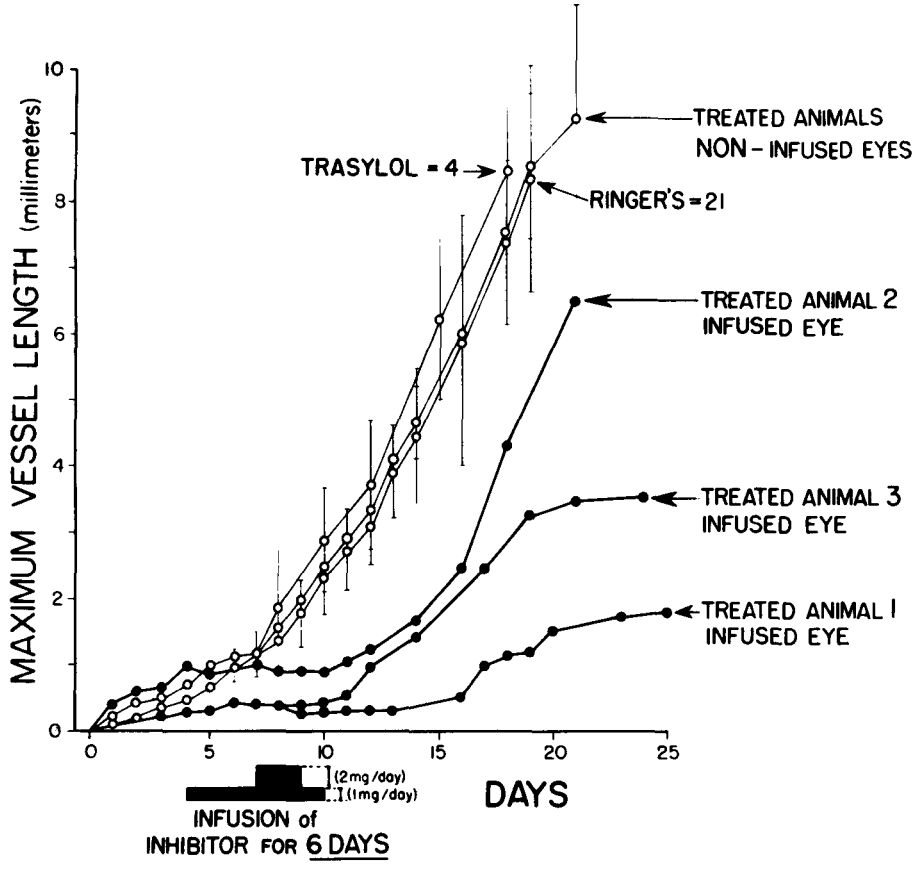

Fig. 5. Infusion of the inhibitor and controls through the right carotid artery in rabbits carrying V2 carcinoma in each cornea (*32*).

treated tumors weighed 0.19 ± 0.06 mg (Fig. 6). Histological examination showed extensive vascular proliferation in controls and nearly none in the treated mice (*32*).

In experiments with both mice and rabbits, animals receiving inhibitor appeared healthy. Histology of major organs and standard blood tests did not indicate differences from normal animals (*32*).

V. Purification

The current purification procedure is as follows: Connective tissue is removed from fresh veal scapular bones. The half of cartilage furthermost from the bone is excised. Cartilage slices (2.0 kg), are extracted in 20 L of 1.0*M* guanidine-HCl and 0.02*M* sodium maleate buffer (pH 6) for 24 h at 25°C. This solution is filtered and then concentrated to 500 mL in a Millipore ultrafiltration unit containing a PTGC cassette (nominal molecular weight limit, 10,000). The retentate is dialyzed against water for 12 h at 4°C and then desalted using the Millipore unit described

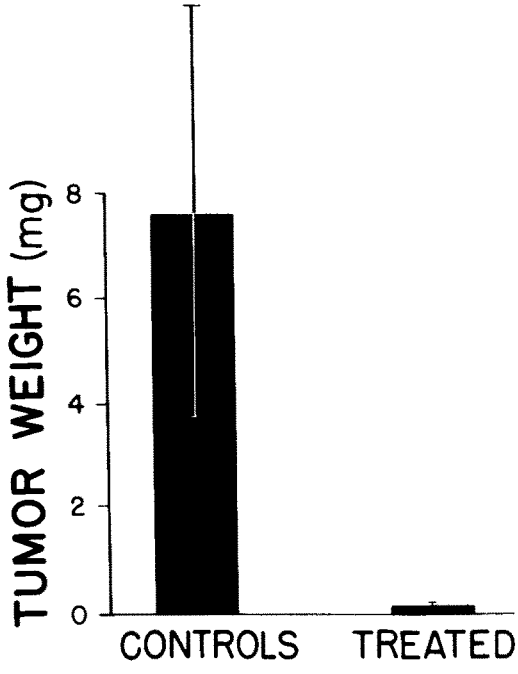

Fig. 6. Weights of B16 melanoma implants in control and treated C57 black/6J mice carrying bilateral B16 melanoma implants at day 7 (*32*).

above. The material is then lyophylized, and chromatographed on a trypsin-Sepharose affinity column. The material eluted at pH 2 is frozen, lypohilized, dissolved in water, dialyzed exhaustively against water at 4°C, passed through a 0.45 μm Millipore filter, and relyophilized. This fraction contains trypsin inhibitory activity, collagenase inhibitory activity, and antiangiogenesis activity, as measured by the inhibition of tumor-induced vascularization in the rabbit cornea. Analysis by polyacrylamide gel electrophoresis of this material reveals a heterogeneous pattern. Polypeptides are separated on the gel into regions designated as A, B, and C (Fig. 7). Fractions A and B are heterogeneous while Fraction C appears to be homogeneous. When electrophoresed on SDS gels, Fraction A migrates as 5–7 bands from 50,000 to 70,000 daltons. Fraction B migrates as 4–6 bands from 14,000 to 28,000 daltons. Fraction C migrates as a single band below 12,000 daltons. When the fractions were assayed for trypsin inhibitory activity, Fraction A was inactive while fractions B and C were active. Fraction C had a much greater specific activity than Fraction B. When assayed for antiangiogenesis activity in the rabbit cornea, Fraction A appeared inactive. Fraction B appeared highly active (80% inhibition of maximum vessel growth rate when compared to controls). Fraction C has not been tested because it contains insufficient material. Further analysis by preparative isoelectric focusing in the presence of urea, of the material that binds to Sepharose-trypsin, reveals two fractions of trypsin-inhibitory activity. One fraction is anionic with isoelectric points between 4.5 and 6.0. The other is cationic with isoelectric points between 9.7 and 10.5 (Fig. 8). The anionic fraction corresponds to region B of the polyacrylamide gel becasue analysis of the B region by analytical isoelectric

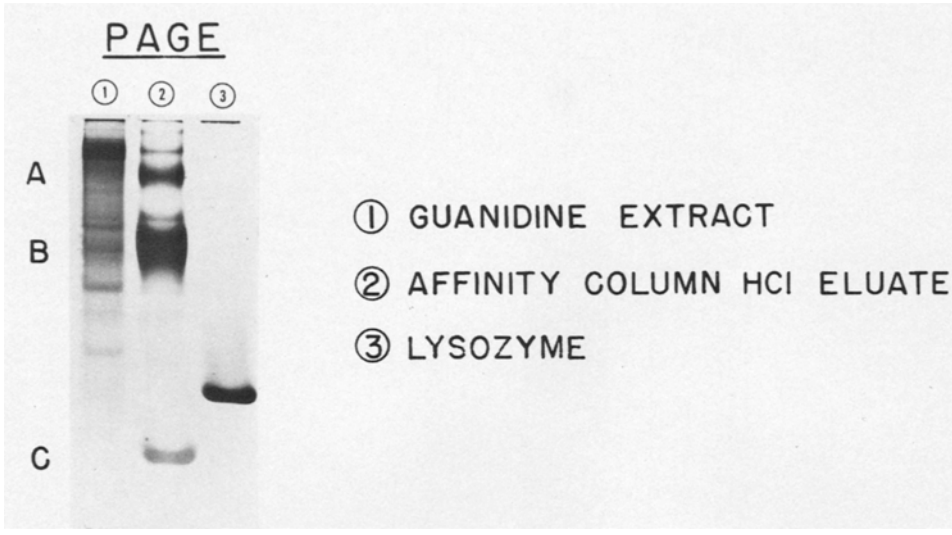

Fig. 7. Polyacrylamide gel electrophoresis (PAGE) using pH 3.2, 6.25*M* urea (*63*).

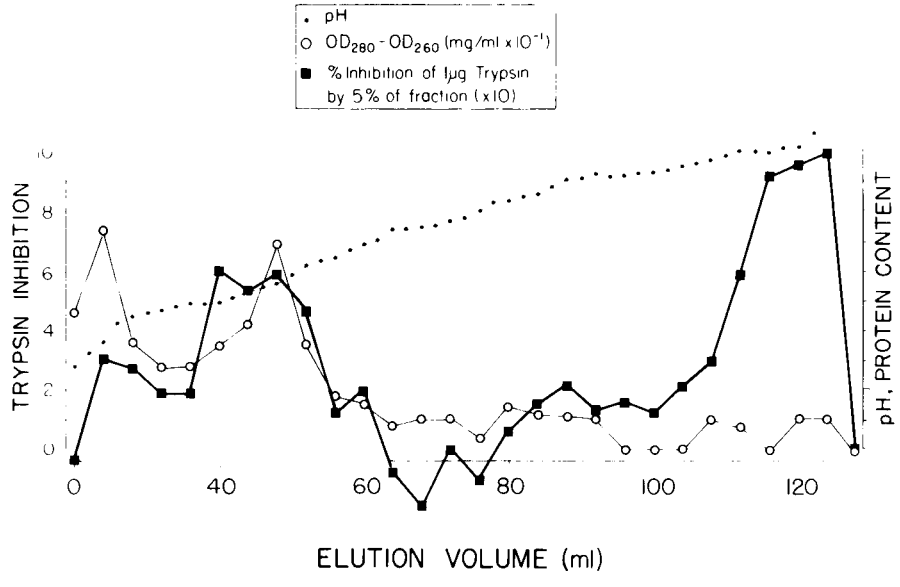

Fig. 8. Preparative isoelectric focusing of trypsin column eluate on LKB 8100 column using ampholytes in the pH 3.5–10 region.

focusing indicates that all the polypeptides have isoelectric points between 4.5 and 6.5 (Fig. 9). The cationic fraction probably corresponds to the homogeneous polypeptide of region C. The region C polypeptide has an almost identical Rf to trasylol, whose isoelectric point is 10.5. Several studies have provided evidence

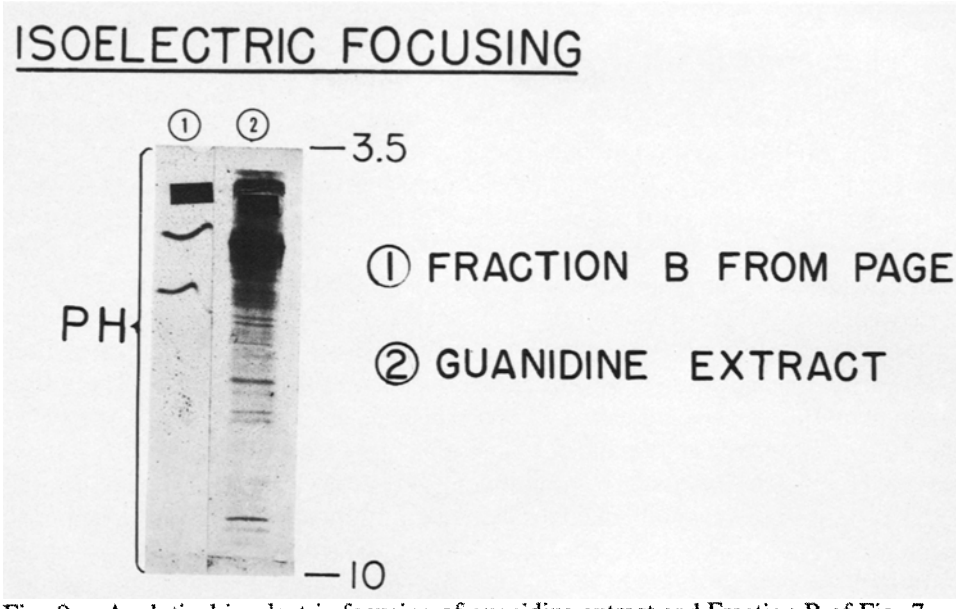

Fig. 9. Analytical isoelectric focusing of guanidine extract and Fraction B of Fig. 7.

based on molecular weight, amino acid composition, and antigenicity that the region C polypeptide from bovine nasal cartilage is trasylol (*34, 35*).

Both the trypsin inhibitory activity and the antiangiogenesis activity of the trypsin-Sepharose eluate are stable to harsh treatment. Exposure to pH 2 during affinity chromatography and to 6*M* urea during polyacrylamide gel electrophoresis have no adverse effect on either of the biological activities. In addition, trypsin inhibitory activity is not significantly altered by exposure to 4*M* guanidine hydrochloride, heating to 100°C for 15 min, or freezing.

Because of the time-consuming nature of preparative polyacrylamide gel electrophoresis, and the apparent stability of both the trypsin-inhibitory and antiangiogenesis activities to harsh conditions, we used high pressure liquid chromatography in 4*M* guanidine hydrochloride as a separation step. When the trypsin affinity eluate was applied to such a column, the trypsin inhibitory activity eluted as a compound of 6000 daltons. Upon SDS gel electrophoresis, it migrated identically to trasylol. Although the fraction containing trypsin inhibitory activity was not homogeneous, but contained some minor bands of higher molecular weight, repeated runs on the HPLC seemed the best approach to preparing enough of the high specific activity trypsin-inhibitory material for testing in the rabbit cornea assay. When this objective was achieved, the material did not appear to have antiangiogenesis activity. Rather, a higher molecular weight fraction of nearly 30,000 daltons displayed activity. We are currently trying to assess what other sorts of activities this fraction may contain. By so doing, we hope to develop a purification scheme that will be more effective than the use of the trypsin-affinity step.

VI. Other Inhibitors

A number of other inhibitors of angiogenesis have recently been identified. One such inhibitor exists in the vitreous humor, which, like cartilage, is vascularized during embryogenesis, but becomes avascular as development continues. When extracts of vitreous humor from rabbits were tested in the rabbit cornea assay (*36, 37*), and on the chick CAM (*38*), angiogenesis was inhibited. Vitreous humors from bovine and human sources have shown similar inhibitory effects (*39*).

Eisenstein et al. have reported the presence of an angiogenesis inhibitor in extracts from aorta. The active material was obtained via 1*M* guanidine extraction followed by passage through 50,000 and 10,000 mw cutoff membranes. The active material inhibits trypsin and collagenase and appears as two major bands of molecular weight 11,000 or less on an electrophoretic gel (*40*). Rifkin and Crowe have provided evidence that the trypsin inhibitor (*41*) is trasylol (*34*). Eisenstein's extract has been shown to inhibit neovascularization stimulated by silver nitrate burns in the rabbit cornea (*42*) as well as the growth of two animal tumors (breast carcinoma, fibrosarcoma) (*40*). The aortic extract also inhibits the growth of aortic endothelial cells in tissue culture (*43*).

Several nontissue-derived inhibitors have also been identified. Gross and coworkers found that the antiinflammatory agents (which are also collagenase inhibitors) medroxyprogesterone and dexamethasone prevent angiogenesis induced by V2 carcinoma, B16 melanoma, and tumor extracts in the rabbit cornea (*44*). In a later study, Bronster et al. found that medroxyprogesterone, but not dexamethosone, inhibited angiogenesis and tumor growth of V2 carcinoma in the rabbit ear (*45*).

Most recently, Taylor and Folkman (*46*) have reported that protamine sulfate is a specific inhibitor of angiogenesis induced by tumors, immune reactions, inflammation, and embryogenesis. Protamine has been effective in inhibiting the growth of fully vascularized subcutaneous tumors of B16 melanoma cells and of lung metastases derived from Lewis lung carcinoma and B16 melanoma cells. However, protamine has proven toxic at high doses and ineffective against other subcutaneous tumors (*46*).

Since protamine can inhibit angiogenesis associated with normal development without the stimulus of tumors, it is likely that its action is specifically targeted at the endothelial cell. Taylor and Folkman suggest that protamine may block the migration of capillary endothelial cells by neutralizing heparin (*46*). Heparin has been shown to stimulate the migration of capillary endothelial cells (*47*). Since capillary cell migration (*48*) and proliferation (*49*) are two critical events in an angiogenic response, any chemical that interferes with either event may cause angiogenesis inhibition. It is possible that protamine may interfere with cell migration. However, it is also possible that protamine or other inhibitors could interfere with the biochemical steps underlying cell migration and proliferation, e.g., the elaboration of enzymes by the growing capillary tip to enable invasion of the connective tissue matrix. The ability to inhibit collagenase, which is both activated and released in the presence of heparin (*50*), may be a critical factor. Collagenase inhibitory activity is common to cartilage and aortic extracts (*51*) as well as dexamethasone (*44*),

medroxyprogesterone (*44*), and protamine (*52*). At present, it is not clear by what mechanism angiogenesis inhibition is achieved and it is possible that there are several steps in a cascade of events leading to neovascularization where intervention may be possible.

VII. Future Directions

Two factors limiting the rate at which purification of tissue-derived angiogenesis inhibitors progresses are the lack of a substantial source of the inhibitor and the lack of an accurate and sensitive assay. To obtain increased amounts of the cartilage-derived inhibitor we are exploring both tissue culture approaches for the large-scale growth of chondrocytes to produce the inhibitor and are searching for sources of cartilage that may contain large amounts of the inhibitor. An interesting recent finding was that cartilage from sharks whose endoskeleton is entirely cartilagenous contains an angiogenesis inhibitor (*53*).

Two types of assays seem promising: (1) Those based on inhibition of capillary endothelial cell migration and (2) those based on inhibition of capillary endothelial cell proliferation. To study migration, we have used a phagokinetic track assay. It has been observed that sparsely plated capillary endothelial cells in Dulbecco's medium with calf serum show little movement over an 18 h period. In contrast, when tumor-conditioned medium replaced Dulbecco's medium, capillary endothelial cells migrate extensively (*54*). If colloidal gold-coated cover plates are used, the area of migration can be quantitated by measuring areas where gold has been swept away by migrating endothelial cells. Preliminary results have shown that the cartilage factor causes a dose-dependent inhibition of capillary endothelial cell migration, but not the migration of other cells including aortic endothelial cells. In addition, other proteins and polysaccharides do not cause inhibition of capillary endothelial cell migration. However, we have found that numerous protease inhibitors that do not inhibit angiogenesis also retard migration.

Another approach to studying the effects of the inhibitor on migration of capillary endothelial cells may be possible. Liotta et al. have developed a method for isolating large areas of intact basement membrane from amnion (*55*). They have shown that various cell types can migrate through the basement membrane with appropriate stimulus. We are currently setting up an assay to look at the ability of capillary endothelial cells to migrate through basement membrane in the presence and absence of the cartilage-derived inhibitor. Basement membrane is a suitable substrate since it consists primarily of Type IV collagen and proteoglycan and underlies blood vessels. Endothelial cells probably must transverse basement membrane in order to penetrate the corneal matrix that consists mainly of Type I collagen (*56*).

A second approach is to look for agents that specifically inhibit the proliferation of endothelial cells. Sorgente and Dorey have reported that extracts from cartilage, but not those that contain the trypsin inhibitory activity, can inhibit aortic endothelial cell growth (*57*). Capillary endothelium, which is directly involved in the angiogenic process, may be an even more desirable cell type with which to test the

effect of cartilage extracts (*58*). An important concern is distinguishing between a genuine inhibitory effect and toxicity. Even if an effective in vitro assay is developed, it will probably be most useful as a screening procedure because it is not clear to what extent in vitro phenomena will be comparable to in vivo results.

The mechanism by which angiogenesis is inhibited by extracts from cartilage and other sources is unknown. Although it is not certain how an angiogenesis inhibitor would be used therapeutically, we have suggested that it could be given after excision of a primary tumor to prevent metastatic foci from becoming vascularized or used against primary tumors, as in the protamine studies (*46*), in particular against highly vascularized yet inoperable tumors (e.g., brain tumors). In addition, it is possible that angiogenesis inhibitors could be used as an adjunct to chemotherapy or immunotherapy (*59*). The potential value of angiogenesis inhibitors may not be limited to cancer therapy. Several other diseases including diabetic retinopathy (*38*), psoraisis (*60*), and forms of arthritis or bone disease (*61*) also exhibit abnormal neovascularization. Angiogenesis inhibitors may prove useful in the treatment and understanding of these diseases. Because endothelial cells rarely proliferate (*62*) except in such disease states (*49*), it is unlikely that these inhibitors would have a deleterious effect on the existing vasculature. Studies to date have shown that angiogenesis inhibitors can prevent neovascularization without damage to existing vessels and organs and without apparent toxicity to the host (*30, 32*). It is conceivable that, as the chemistry of angiogenesis inhibitors becomes clear, a new class of pharmaceuticals with the capability of interfering with neovascularization will be produced and a greater understanding of the factors underlying the complex process of vascular proliferation will result.

Acknowledgments

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