Journal of Cellular Biochemistry 58:199–207 (1995)

ARTICLES

Suramin Inhibits C6 Glioma-Induced Angiogenesis In Vitro

Brenda L. Coomber

Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Abstract Aspects of tumor-induced angiogenesis in vitro were examined using an assay involving collagen gel invasion by a surface monolayer of bovine endothelial cells under the influence of serum free conditioned medium produced by C6 cells, an experimentally derived rat glial tumor cell line. The effects of the polyanionic compound suramin, known to interfere with growth factor/cell signaling on this process were evaluated. Collagen gel invasion was quantified by adding C6 conditioned medium with or without various doses of suramin to monolayers of bovine aortic endothelial cells grown on type I collagen gels in transwell inserts. Cultures were monitored with phase-contrast microscopy. After various periods of incubation collagen gels were fixed, embedded in epoxy resin, and 1-μm thick sections were stained with toluidine blue. Additional cultures were used to evaluate the effects of C6 conditioned medium and suramin on endothelial cell proliferation, and on chemotaxis through 8-μm pores. C6 glioma cell conditioned medium induced large vessel endothelial cells to sprout into the underlying collagen matrix and subsequently form networks of capillary like tubes. Conditioned medium was also chemotactic and mitogenic for these cells. The addition of suramin to C6 glioma conditioned medium prevents tube formation in collagen gels, and inhibits both endothelial cell proliferation and chemotaxis in a dose dependent manner. These results suggest that glial tumor cell conditioned medium induces angiogenesis in large vessel endothelial cells in vitro via mechanisms which are disrupted by suramin, most likely involving tumor-derived growth factor release and/or endothelium-mediated matrix proteolysis.

Key words: endothelium, cancer, tube formation, collagen gel

Angiogenesis plays an important role in normal and in pathological processes during responses to tissue damage such as wound healing, tumor growth, and metastasis [Folkman and Shing, 1992]. The angiogenic response requires that endothelial cells within the vessel wall alter their phenotype to one capable of directed cell migration and proliferation. These cells invade the extracellular matrix and proliferate to produce several vascular sprouts consisting of solid cords which connect to form loops. Eventually, a lumen forms in these solid cords of endothelia and blood flow is reestablished through the new vessel [Ausprunk and Folkman, 1977].

A variety of growth factors and cytokines have been implicated in aspects of the angiogenesis response. These include, but are not restricted to, basic fibroblast growth factor (bFGF), vascular endothelial growth factor/vascular permeability factor (VEGF), platelet-derived growth factor (PDGF), scatter factor, TGF-α and TGF-β, epidermal growth factor (EGF), insulin like growth factors (IGFs), and TNF-α [Folkman and Shing, 1992]. Many of these factors, such as bFGF and VEGF [Goto et al., 1993; Pepper et al., 1993, 1994], scatter factor [Rosen et al., 1993], and EGF [Goldman et al., 1993] stimulate angiogenic behaviour such as endothelial cell motility and matrix invasion in vitro, as well as being angiogenic in vivo. Suramin, which inhibits angiogenesis on chicken chorioallantoic membrane (CAM) assays [Gagliardi et al., 1992], is known to inhibit or interfere with the actions of numerous potential angiogenic substances, including PDGF, bFGF, TGF-β, EGF, and IGFs [Eisenberger and Fontana, 1992], and has been reported to prevent bFGF induced angiogenesis in vitro [Pesenti et al., 1992].

Considerable effort has been expended developing and characterizing in vitro systems that

Received September 27, 1994; accepted October 18, 1994.

Address reprint requests to Brenda L. Coomber at Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

accurately model aspects of the angiogenic response. Directed cell migration toward a specific stimulus (chemotaxis) is likely involved in recruiting endothelial sprouts during angiogenesis in vivo [Auerbach et al., 1991; Folkman and Shing, 1992]. Endothelial proliferation and migration is considered to be required for "early" aspects of the angiogenic response, while morphological alterations that lead to lumen formation are considered aspects of the "later" response to angiogenic stimuli [Yang and Moses, 1990]. These "later" aspects can be modeled by culturing endothelial cells with exogenous extracellular matrix components. Basal lamina constituents, notably collagen type IV and laminin, and reconstituted basal lamina prepared from Engelbreth-Holm-Swarm tumor epithelial cells (Matrigel) rapidly induce endothelial cells to form tubes when grown on or within gels composed of these matrixes [Kubota et al., 1988; Schnaper et al., 1993; Marx et al., 1994]. Other extracellular components, such as interstitial collagens (types I and III) and fibronectin, also have limited ability to induce such reorganization [Vernon et al., 1992; Nguyen et al., 1993; Marx et al., 1994]. Cytokines with putative angiogenic properties, such as TNF-α [Niedbala and Picarella, 1992], TGF-β [Madri and Marx, 1992], PDGF [Battegay et al., 1994], and bFGF [Pepper et al., 1990; Madri and Marx, 1992] will enhance endothelial tube formation in cells cultured on or within polymerized collagen, fibrin, or Matrigel.

While these types of assays can provide considerable information about matrix–endothelial cell interactions which may lead to morphological reorganization, they do not accurately mimic the "sprouting" of capillary buds seen during angiogenesis in vivo. Long-term cultures of endothelial monolayers derived from both large and small vessels will occasionally produce spontaneous "sprouting" cells which migrate into the underlying matrix [Montesano et al., 1983; Olander et al., 1985]. As it is thought that sprouting will only occur when sufficient suitable matrix material has been secreted by the cultured cells, this process can be enhanced by growing monolayers of endothelial cells on preformed matrix material.

Tumor cells in vitro produce as yet uncharacterized factors that induce angiogenic processes such as tube formation in a collagen gel [Niida et al., 1992; Abe et al., 1993] and enhance endothelial chemotaxis [Takaki et al., 1992]. Tumor cells can induce angiogenic behaviour in cultured endothelial cells by releasing chemotactic factors [Augustin-Voss and Pauli, 1992; Takaki et al., 1992]. Glioma cells will also induce capillary-like tube formation in cocultured microvascular endothelium [Laterra et al., 1994]. Cytokines and growth factors previously implicated in the angiogenic response have recently been detected in human and experimental animal tumors in vivo and in vitro. Substances such as bFGF [Tanimoto et al., 1991; Brem et al., 1992] and VEGF [Plate et al., 1992] have been localized to cells in tumor tissue at higher concentrations than that found in equivalent normal tissue. In vitro studies have confirmed the potential role of these angiogenic substances in tumor vascularization. Substances known to interfere with growth factor activity, such as antiangiogenic heparin binding steroids and suramin, will also inhibit tumor cell [Minniti et al., 1992; Zugmaier et al., 1992], and endothelial cell proliferation in vitro [Wade et al., 1992], as well as reduce experimental tumor growth in vivo [Pesenti et al., 1992; Zugmaier et al., 1992].

This study examines the ability of suramin to inhibit in vitro angiogenesis (endothelial tube formation, proliferation, and chemotaxis) induced by C6 glioma serum free conditioned medium.

METHODS

Cells and Conditioned Media

Bovine aortic endothelial cells (BAEC) were isolated via collagenase digestion of thoracic aortas [Coomber and Gotlieb, 1990], obtained from animals less than 2 years of age. Cells were confirmed as endothelial by phase-contrast morphology, Factor VIII related antigen staining, and uptake of fluorochrome-labeled acetylated LDL (Molecular Probes, Eugene, OR). C6 glioma cells are CCL 107 from ATCC, a rat cell line originally derived from brains of carcinogen treated animals. All cells were propagated in MEM containing gentamycin and 10% FBS (Canadian Life Technologies, Burlington, Ontario, Canada), and cultured in a humidified atmosphere at 38°C, 5% CO_2. For these studies, BAEC were used between passages 3 and 8. Conditioned media (CM) was produced from postconfluent C6 cells by incubating them for 24 h in MEM containing 0.1 mg/ml BSA. After incubation, CM was collected, centrifuged to remove any cells, pooled, and stored in aliquots at −80°C. Suramin (Sodium Suramin, Miles Inc., West Haven, CT) was added to freshly thawed CM at concentrations ranging from 0.1 to 1 mM. Media

for control assays consisted of MEM plus 0.1 mg/ml BSA.

Collagen Gel Angiogenesis

Sterile solutions of unpolymerized collagen I (*Cellagen,* ICN Flow, Mississauga, Ontario, Canada) were mixed with concentrated culture media and buffers on ice to produce a final collagen concentration of 2 mg/ml. Cold unpolymerized collagen solution was added to collagen transwell inserts (12-mm diameter, 0.4-μm pores; Costar, Cambridge, MA) and polymerized at 38°C. The resultant hydrated gel was covered with culture media containing sufficient endothelial cells to form a confluent monolayer and incubated for 24 h. Serum free media, or serum free C6 CM with or without suramin, was then added to both compartments of the transwells. Cultures were monitored by phase-contrast microscopy and fed appropriate media every 2–3 days. After 7 days in culture, collagen gels were fixed with 2.5% gluteraldehyde and 1% OsO_4, and embedded in epoxy resin. Sections 1 μm thick, stained with toluidine blue, were examined to evaluate the extent of matrix invasion and tube formation by surface endothelial cells.

Proliferation and Migration

The effects of C6 CM and suramin on endothelial cell proliferation were determined by quantifying [^{3}H]thymidine incorporation [Coomber, 1991]. Briefly, endothelial cells were plated into 96-well culture dishes at subconfluent densities in MEM plus 10% FBS and incubated for 24 h. Media was then replaced with control media, or test media containing suramin, and 2 μCi [^{3}H]thymidine/ml and incubated for 24 h. Cells were harvested onto glass fibre mats using an LKB harvester and mats were counted in a 1205 Betaplate flatbed scintillation counter (LKB, Turku, Finland). Results are expressed as the mean of at least triplicate experiments. The effects of C6 CM with or without suramin on endothelial cell proliferation were determined using a modification of the crystal violet assay [Keisari, 1992]. Endothelial cells at low density were seeded into multiwell plates in MEM plus 10% FBS. After 24 h, media was replaced with control media, or test media containing suramin, and cultures were incubated for a further 24 h. Cells were then fixed, stained with 0.5% crystal violet in methanol, extracted with 10% acetic acid, and the absorbance of each well was read at 620 nm. Absorbance levels were compared to those obtained on a standard curve produced from known numbers of endothelial cells. Results are expressed as the mean of at least triplicate experiments.

Endothelial migration was assessed using a modified Boyden chamber technique. Costar transwell inserts, 6.5-mm diameter with 8-μm pores, were used. Endothelial cell suspensions consisting of 5×10^5 cells in 100 μl control media were added to 6.5-mm-diameter inserts, and 600 μl of test media was added to the lower compartment. After incubation for 16 h at 38°C inserts were fixed in 3% paraformaldehyde, the interior surface of the insert was scraped to remove any remaining cells, then inserts were stained with toluidine blue, rinsed and air dried. Filters were cut from inserts, mounted and viewed with a Zeiss standard microscope. Ten 100× oil immersion fields were counted from each filter, and the mean cell number/field was calculated from at least three separate experiments.

Statistical Analysis

Results from individual experiments were pooled and means and standard deviations calculated. One way analysis of variance (ANOVA) and Student's *t*-test were used to detect differences in cell proliferation and cell migration between and within experimental groups, using a 5% level of significance.

RESULTS

This study demonstrates that serum free conditioned media produced by C6 glioma cells will induce angiogenic behaviour in bovine large vessel endothelial cells in an in vitro assay. Endothelial cells cultured on collagen I gels in the presence of C6 CM invade the underlying gel and form networks of cords and tubes within the gel below the plane of the monolayer surface (Fig. 1B). Occasional lucent areas can be seen in phase contrast, which apparently correspond to the development of lumina within these cells, thus giving rise to capillary-like tubes; 1-μm-thick sections of epoxy resin-embedded gels show numerous sprouting endothelial cells under the surface monolayer and sections through putative capillaries that clearly contain lumen-like cavities (Fig. 2A,B). The dimension of these capillary-like structures is about 8 μm in diameter, an appropriate size for capillary blood vessels in vivo [Coomber et al., 1988].

This monolayer reorganization and capillary sprouting occurs rapidly, with obvious invasion of the collagen gel occurring by 3 days, and

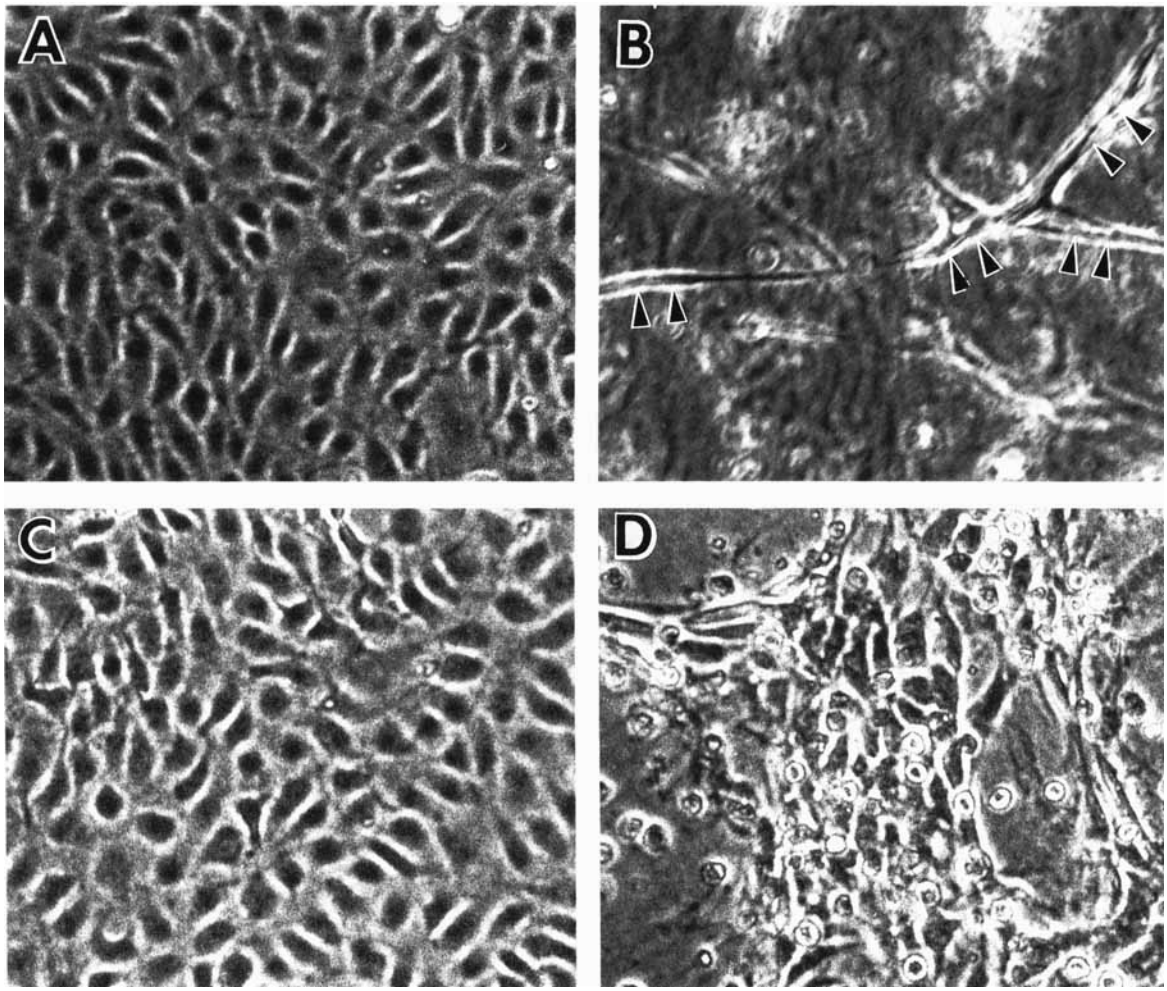

Fig. 1. Phase-contrast images of endothelial monolayers grown on collagen gels for 7 days. **A:** Cells cultured with serum free medium remain as a confluent monolayer of the surface of the collagen gel. **B:** In the presence of serum free C6 glioma conditioned medium (C6 CM) extensive sprouting into the gel, and cord and tube formation by endothelial cells can clearly be seen (arrowheads). **C,D:** Similar cultures treated with serum free C6 CM containing suramin for 7 days. Cultures treated with C6 CM plus 0.5 mM suramin remain as confluent monolayers on the surface of the gel (C), while a higher dose of suramin (1 mM) leads to monolayer disruption and no obvious sprouting, cord, or tube formation (D). ×280.

well-formed networks of cords and sprouts by seven days incubation with serum free C6 CM. Control cultures incubated with serum free media alone do not show any appreciable sprouting into the underlying collagen gel, and tube formation is not seen (Fig. 1A). Incubation with media containing 10% FBS also did not induce matrix invasion or tube formation. Addition of the polyanionic substance suramin to this assay system prevents endothelial sprouting and tube formation induced by C6 CM. Cultures treated with serum free C6 CM containing 0.5 mM suramin remain as confluent monolayers on the collagen gel surface, while treatment with 1 mM suramin causes considerable monolayer disruption and cell retraction, but no obvious gel invasion or tube formation (Fig. 1C,D).

As well as the morphologic modifications seen above, the angiogenic response may also involve endothelial cell proliferation and directed migration. Serum free C6 CM is mitogenic for bovine large vessel endothelial cells, with significant increases in [^{3}H]thymidine incorporation, and cell number as indicated by crystal violet staining intensity, over responses seen with serum free media alone (Fig. 3). Addition of suramin (0.5 or 1 mM) to serum free conditioned media significantly reduces the proliferative response to control levels in a dose dependent fashion (Fig. 3).

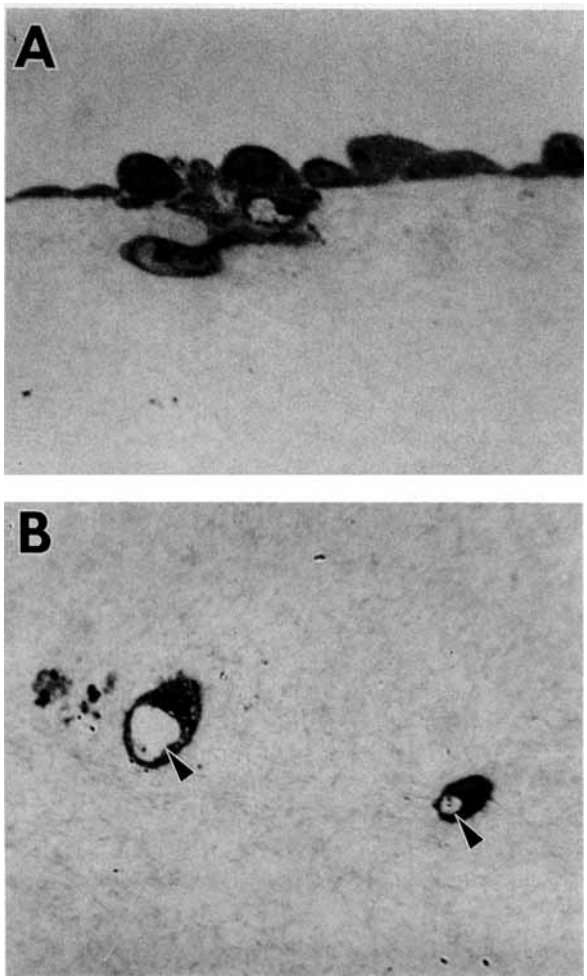

Fig. 2. One μm toluidine blue-stained sections of endothelial cells grown on collagen in the presence of serum free C6 CM for 7 days. **A:** Some areas of gels show accumulations of endothelial cells invading the gel underlying the monolayer. **B:** Tubes of endothelial cells containing obvious capillary like lumens (arrowheads) are also seen deep to the surface monolayer in cultures treated with serum free C6 CM. ×875.

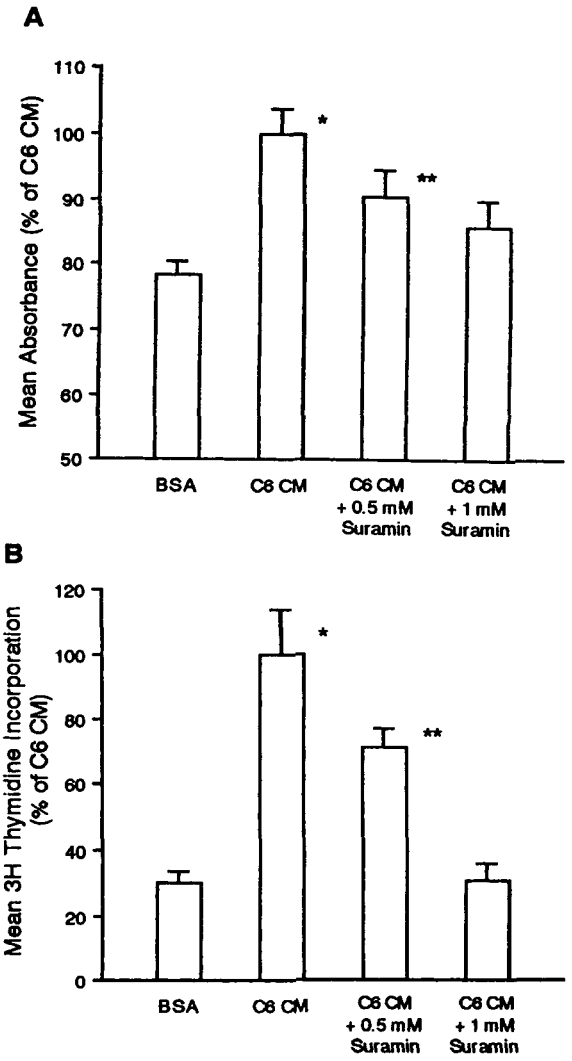

Fig. 3. Endothelial cell proliferation as measured by crystal violet staining intensity (**A**) and [^{3}H]thymidine incorporation (**B**) in cultures treated with serum free medium (BSA), C6 CM, and C6 CM containing suramin for 24 h. Significant enhancement of cell proliferation was seen with C6 CM over all other treatments (*$P < 0.05$). C6 CM plus 0.5 mM suramin significantly increased cell proliferation over C6 CM plus 1 mM suramin, or BSA control (**$P < 0.05$).

Bovine large vessel endothelial cells respond to serum free C6 CM by migrating through pores in a two-chamber assay system (Fig. 4). Serum free medium alone had no effect on endothelial cell motility. Addition of suramin (0.1–1 mM) to serum free C6 conditioned medium significantly reduces this chemotactic response in a dose-dependent fashion (Fig. 4).

DISCUSSION

Rat C6 glioma cells will form rapidly growing well-vascularized tumors in vivo when implanted into brain or other sites [Coomber et al., 1988; Plate et al., 1993; Trojan et al., 1993]. This study demonstrates that serum free conditioned media from these C6 glioma cells is able to induce in vitro angiogenic behaviour (cell proliferation, migration, and tube formation in collagen gels) in endothelium derived from bovine aorta. This is in agreement with other studies using this C6 glioma, and with other in vitro experiments involving different rat and human glial cell tumors, which also report their ability to induce angiogenesis in vitro. However, these previous studies describing tumor induced angiogenic behaviour differ from the present study, in that they use tumor cells and *endothelium from*

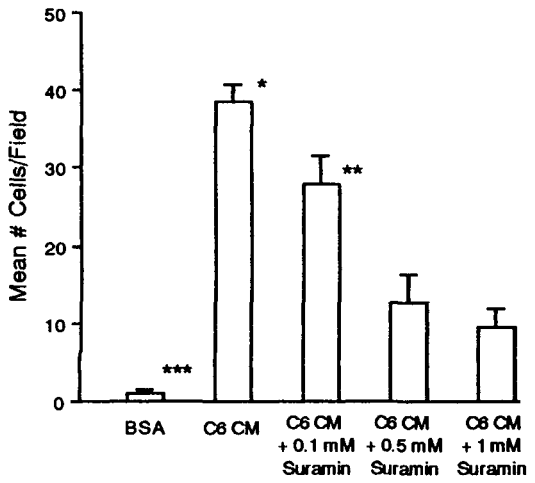

Fig. 4. Endothelial chemotaxis through 8 μm diameter pores after 16-h incubation with serum free media (BSA), C6 CM, or C6 CM containing suramin. Significantly highest chemotactic response was seen in C6 CM-treated cultures (*$P < 0.05$), followed by cultures treated with C6 CM and 0.1 mM suramin (**$P < 0.05$). C6 CM containing higher doses of suramin had lower numbers of migrating cells, but chemotactic responses were significantly higher than for BSA controls (***$P < 0.05$).

the same species, use microvessel endothelium, require the co-culture of tumor cells and endothelium or are performed in the presence of serum [Niida et al., 1992; Abe et al., 1993; Laterra et al., 1994]. Many assays of endothelial "capillary" formation in vitro use a basal lamina-derived product produced by Engelbreth–Holm–Swarm tumor cells known as Matrigel. This matrix, especially rich in laminin and collagen IV has been shown to induce rapid morphological alterations in cultured endothelial cells, specifically the formation of networks of cords or tubes on the surface or within the matrix [Gerritsen et al., 1993; Schnaper et al., 1993; Marx et al., 1994]. However, Matrigel preparations are known to be contaminated with such growth factors as TGF-β, bFGF, and IGF-I, as well as matrix degrading proteases [Vukicevic et al., 1992; Santos and Nigam, 1993; Schnaper et al., 1993]. As these factors have angiogenic activity in vitro, the results of earlier work using Matrigel matrix must be interpreted with caution [Vukicevic et al., 1992]. Thus, the assay system described here using serum free CM, collagen I gels, and heterologous sources of endothelium and tumor cells is potentially more flexible and versatile, and will be useful for examining aspects of tumor induced angiogenesis common to many systems.

Tumor cells are known to produce and secrete a variety of factors which could contribute to this angiogenic response. The present study did not attempt to isolate nor characterize which factor(s) produced by rat C6 glioma cells are inducing the observed angiogenic response, but previous studies would suggest that bFGF, IGF-I, and VEGF are likely potential angiogenic factors, as these have been implicated in numerous angiogenic assays and are produced by these C6 glioma cells [Westermann and Unsicker, 1990; Plate et al., 1993; Trojan et al., 1993]. Preliminary studies in my laboratory using neutralizing antibodies to bFGF, however, were not able to suppress this angiogenic response produced by serum free C6 CM (unpublished results). It is possible that bFGF may be acting with other glioma derived factors, such as IGF-1, VEGF, EGF, or TGF-β [Pepper et al., 1992; Goldman et al., 1993; Goto et al., 1993; Plate et al., 1993] or that these other cytokines alone may be responsible for the angiogenesis produced in this assay, as has been shown in other systems [Vukicevic et al., 1992; Gajdusek et al., 1993].

Cell migration is important for in vivo angiogenesis as endothelial cells which have sprouted from existing microvessels will migrate through the connective tissue toward a chemotactic stimulus, such as a nidus of cancer cells [Folkman and Shing, 1992]. Tumor cells can induce angiogenic behaviour in cultured endothelial cells by releasing such chemotactic factors [Augustin-Voss and Pauli, 1992; Takaki et al., 1992]. The strong chemotactic response seen in this study with C6 CM relative to serum free media is consistent with this requirement. However, endothelial cell proliferation, although significantly enhanced by C6 CM, is still less than a twofold increase in this study. Conditioned media from C6 glioma also has been shown to inhibit rat microvessel endothelial cell proliferation [Niida et al., 1992]. The role of endothelial cell proliferation during angiogenesis is unclear, as some reports indicate enhanced DNA synthesis during angiogensis in vitro [Battegay et al., 1994], while others show that proliferation is a relatively minor component of endothelial cord and tube formation in vitro [Ingber and Folkman, 1989] and may also not be a major controlling factor during tumor angiogenesis in vivo [Fox et al., 1993]. Since some angiogenic factors that are chemotactic for endothelium are not mitogenic to these cells [D'Amore, 1992], mul-

tiple constituents in the serum free C6 CM may be inducing the angiogenesis seen in this study.

Suramin has been previously shown to be able to inhibit tumor cell and growth factor-induced angiogenesis in vivo and in vitro [Gagliardi et al., 1992]. Suramin is known to modulate the activity of numerous growth factors, either by complexing with soluble factors or by interfering with growth factor receptor interactions [Eisenberger and Fontana, 1992]. Factors likely involved in the angiogenic response such as IGF-I, bFGF, or TGF-β are interfered with by suramin in this manner [Pesenti et al., 1992]. Studies suggest that suramin is able to form extremely stable complexes with such factors [Lopez Lopez et al., 1992; Middaugh et al., 1992; Stein, 1993; Braddock et al., 1994], and previous work in this laboratory indicates that excess exogenous bFGF is not able to ameliorate the affects of suramin on cultured endothelial cells [Coomber, 1993]. It is therefore likely that the inhibition of angiogenic response seen in the present study is due to suramin-mediated modulation of C6 glioma-derived growth factor availability to or interaction with endothelial cells.

Endothelial cell invasion of the surrounding stroma, a characteristic feature of angiogenesis in vivo, requires matrix proteolysis [Folkman and Shing, 1992; Matrisian, 1992]. Numerous metalloproteinases, specifically collagenases, gelatinases, and stromelysins are involved in the degradation of basal lamina components, interstitial collagens, and proteoglycans that occurs as endothelial cells sprout away from existing vessels and migrate through the surrounding connective tissue. In addition, plasminogen activators, specifically uPA, are able to proteolytically activate plasminogen to the general protease plasmin, which can further activate matrix metalloproteinase pro-forms, as well as initiate matrix component degradation. There is a positive correlation between matrix proteolysis and proteolytic enzyme production in vitro, and tumor growth and spread in vivo [Kataoka et al., 1993; Montgomery et al., 1993; Shaughnessy et al., 1993]. Cord formation by endothelial cells cocultured with C6 glioma requires uPA activity [Laterra et al., 1994]. Plasminogen activator production is induced by bFGF in cultured endothelial cells, and acts with TGF-β to produce the balance in matrix proteolysis apparently essential for capillary morphogenesis in vitro [Flaumenhaft et al., 1992; Pepper and Montesano, 1992]. It is therefore possible that the angiogenic response seen in this study is, at least partially, due to C6-derived factors that enhance endothelial plasminogen activator production, hence matrix proteolysis and cell invasion. Suramin could inhibit the angiogenic response seen by interfering with plasminogen activator activity or binding of plasminogen activator to its cell surface receptor, as has recently been shown [Behrendt et al., 1993; Ellis and Danø, 1993].

The results of this study indicate that serum free media conditioned by rat C6 glioma cells is able to induce angiogenic behaviour (tube formation, proliferation, chemotaxis) in bovine large vessel-derived endothelial cells. This angiogenesis is inhibited by suramin in a dose-dependent fashion. This inhibition is likely due to effects of suramin on angiogenic growth factor(s) present in C6 conditioned medium, or to the ability of suramin to interfere with essential matrix proteolysis, or a combination of these events. The nature of this suramin mediated inhibition of C6 CM induced angiogenesis is currently under investigation.

ACKNOWLEDGMENTS

This study was supported by grants from NSERC Canada and the Banting Research Foundation. The technical assistance of Kanwal Minhas, Paula Krimer, and Robert Laurignano is gratefully acknowledged.

REFERENCES

Abe T, Okamura K, Ono M, Kohno K, Mori T, Hori S, Kuwano M (1993): Induction of vascular endothelial tubular morphogenesis by human glioma cells. Am J Pathol 92:54–61.

Auerbach R, Auerbach W, Polakowski I (1991): Assays for angiogenesis: A review. Pharmacol Ther 51:1–11.

Augustin-Voss HG, Pauli BU (1992): Quantitative analysis of autocrine-regulated, matrix-induced, and tumor cell-stimulated cell migration using a silicon template compartmentalization technique. Exp Cell Res 198:221–227.

Ausprunk DH, Folkman J (1977): Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. Microvasc Res 14:53–65.

Battegay EJ, Rupp J, Iruela-Arispe L, Sage EH, Pech M (1994): PDGF-BB modulates endothelial proliferation and angiogenesis in vivtro via PDGF β-receptors. J Cell Biol 125:917–928.

Behrendt N, Rønne E, Danø K (1993): Binding of urokinase-type plasminogen activator to its cell surface receptor is inhibited by low doses of suramin. J Biol Chem 268:5985–5989.

Braddock PS, Hu D-E, Fan T-PD, Stratford IJ, Harris AL, Bicknell R (1994): A structure–activity analysis of antagonism of the growth factor and angiogenic activity of basic fibroblast growth factor by suramin and related polyanions. Br J Cancer 69:890–898.

Brem S, Tsanaclis AMC, Gately S, Gross JL, Herblin WF (1992): Immunolocalization of basic fibroblast growth factor to the microvasculature of human brain tumors. Cancer 70:2673–2680.

Coomber BL (1991): Cytoskeleton in TGF-β- and bFGF-modulated endothelial monolayer repair. Exp Cell Res 194:42–47.

Coomber BL (1993): Centrosome orientation in regenerating endothelial monolayers requires bFGF. J Cell Biochem 52:289–296.

Coomber BL, Gotlieb AI (1990): In vitro endothelial wound repair: Interaction of cell migration and proliferation. Arteriosclerosis 10:215–222.

Coomber BL, Stewart PA, Hayakawa EM, Farrell CL, Del Maestro RF (1988): A quantitative assessment of microvessel ultrastructure in C6 astrocytoma spheroids transplanted to brain and to muscle. J Neuropathol Exp Neurol 47:29–40.

D'Amore PA (1992): Mechanisms of endothelial growth control. Am J Respir Cell Mol Biol 6:1–8.

Eisenberger MA, Fontana JA (1992): Suramin, an active nonhormonal cytotoxic drug for treatment of prostate cancer: Compelling reasons for testing in patients with hormone-refractory breast cancer. J Natl Cancer Inst 84:3–5.

Ellis V, Danø K (1993): Specific inhibition of the activity of the urokinase receptor-mediated cell-surface plasminogen activation system by suramin. Biochem J 296:505–510.

Flaumenhaft R, Mayumi A, Mignatti P, Rifkin DB (1992): Basic fibroblast growth factor-induced activation of latent transforming growth factor β in endothelial cells: Regulation of plasminogen activator activity. J Cell Biol 118:901–909.

Folkman J, Shing Y (1992): Angiogenesis. J Biol Chem 267:10931–10934.

Fox SB, Gatter KC, Bicknell R, Going JJ, Stanton P, Cooke TG, Harris AL (1993): Relationship of endothelial cell proliferation to tumor vascularity in human breast cancer. Cancer Res 53:4161–4163.

Gagliardi A, Hadd H, Collins DC (1992): Inhibition of angiogenesis by suramin. Cancer Res 52:5073–5075.

Gajdusek CM, Luo Z, Mayberg MR (1993): Basic fibroblast growth factor and transforming growth factor beta-1: Synergistic mediators of angiogenesis in vitro. J Cell Physiol 157:133–144.

Gerritsen ME, Niedbala MJ, Szczepanski A, Carley WW (1993): Cytokine activation of human macro- and microvessel-derived endothelial cells. Blood Cells 19:325–342.

Goldman CK, Kim J, Wong W-L, King V, Brock T, Gillespie GY (1993): Epidermal growth factor stimulates vascular endothelial growth factor production by human malignant glioma cells: A model of glioblastoma multiforme pathophysiology. Mol Biol Cell 4:121–133.

Goto F, Goto K, Weindel K, Folkman J (1993): Synergistic effects of vascular endothelial growth factor and basic fibroblast growth factor on the proliferation and cord formation of bovine capillary endothelial cells within collagen. Lab Invest 69:508–517.

Ingber DE, Folkman J (1989): Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis in vitro: role of extracellular matrix. J Cell Biol 109:317–330.

Kataoka H, DeCastro R, Zucker S, Biswas C (1993): Tumor-derived collagenase-stimulatory factor increase expression of interstitial collagenase, stromelysin, and 72-kDa gelatinase. Cancer Res 53:3154–3158.

Keisari Y (1992): A colorimetric microtiter assay for the quantification of cytokine activity on adherent cells in tissue culture. J Immunol Methods 146:155–161.

Kubota Y, Kleinman HK, Martin GR, Lawley TJ (1988): Role of laminin and basement membrane in the morphological differentiation of human endothelial cells into capillary-like structures. J Cell Biol 107:1589–1598.

Laterra J, Indurti RR, Goldstein GW (1994): Regulation of in vitro glia-induced microvessel morphogenesis by urokinase. J Cell Physiol 158:317–324.

Lopez Lopez R, Peters GJ, van Loenen AC, Piazo PE, van Rijswijk REN, Wagstaff J, Pinedo HM (1992): The effects of schedule, protein binding and growth factors on the activity of suramin. Int J Cancer 51:921–926.

Madri JA, Marx M (1992): Matrix composition, organization and soluble factors: Modulators of microvascular cell differentiation in vitro. Kidney Int 41:560–565.

Marx M, Perlmutter RA, Madri JA (1994): Modulation of platelet-derived growth factor receptor expression in microvascular endothelial cells during in vitro angiogenesis. J Clin Invest 93:131–139.

Matrisian LM (1992): The matrix-degrading metalloproteinases. BioEssays 14:455–463.

Middaugh CR, Mach H, Burke CJ, Volkin DB, Dabora JM, Tsai PK, Bruner MW, Ryan JA, Marfia KE (1992): Nature of the interaction of growth factors with suramin. Biochemistry 31:9016–9024.

Minniti CP, Maggi M, Helman LJ (1992): Suramin inhibits the growth of human rhabdomyosarcoma by interupting the insulin-like growth factor II autocrine growth loop. Cancer Res 52:1830–1835.

Montesano R, Orci L, Vassalli P (1983): In vitro rapid organization of endothelial cells into capillary-like networks is promoted by collagen matrices. J Cell Biol 97: 1648–1652.

Montgomery AMP, De Clerck YA, Langley KE, Reisfeld RA, Mueller BM (1993): Melanoma-mediated dissolution of extracellular matrix: Contribution of urokinase-dependent and metalloproteinase-dependent proteolytic pathways. Cancer Res 53:693–700.

Nguyen M, Strubel NA, Bischoff J (1993): A role for sialyl Lewis-X/A glycoconjugates in capillary morphogenesis. Nature 365:267–269.

Niedbala MJ, Picarella MS (1992): Tumor necrosis factor induction of endothelial cell urokinase-type plasminogen activator mediated proteolysis of extracellular matrix and its antagonism by γ-interferon. Blood 79:678–687.

Niida H, Minakawa T, Tanaka R (1992): Angiogenesis induced by rat glioma cells in vitro. No Shinkei Geka 20: 1169–1172.

Olander JV, Bremer ME, Marasa JC, Feder J (1985): Fibrin-enhanced endothelial cell organization. J Cell Physiol 125:1–9.

Pepper MS, Montesano R (1992): Proteolytic balance and capillary morphogenesis. Cell Diff Dev 32:319–328.

Pepper MS, Belin D, Montesano R, Orci L, Vassalli J-D (1990): Transforming growth factor-beta 1 modulates basic fibroblast growth factor-induced proteolytic and angiogenic properties of endothelial cells in vitro. J Cell Biol 111:743–755.

Pepper MS, Ferrara N, Orci L, Montesano R (1992): Potent synergism between vascular endothelial growth factor and basic fibroblast growth factor in the induction of angiogenesis in vitro. Biochem Biophys Res Commun 189:824–831.

Pepper MS, Vassalli J-D, Orci L, Montesano R (1993): Biphasic effect of transforming growth factor-β_1 on in vitro angiogenesis. Exp Cell Res 204:356–363.

Pepper MS, Wasi S, Ferrara N, Orci L, Montesano R (1994): In vitro angiogenesis and proteolytic properties of bovine lymphatic endothelial cells. Exp Cell Res 210:298–305.

Pesenti E, Sola F, Mongelli N, Grandi M, Spreafico F (1992): Suramin prevents neovascularization and tumor growth through blocking of basic fibroblast growth factor activity. Br J Cancer 66:367–372.

Plate KH, Breier G, Millauer B, Ullrich A, Risau W (1993): Up-regulation of vascular endothelial cell growth factor and its cognate receptors in a rat glioma model of tumor angiogenesis. Cancer Res 53:5822–5827.

Plate KH, Breier G, Weich HA, Risau W (1992): Vascular endothelial growth factor is a potential tumor angiogenesis factor in human gliomas in vivo. Nature 359:845–848.

Rosen EM, Zitnik RJ, Elias JA, Bhargava MM, Wines J, Goldberg ID (1993): The interaction of HGF-SF with other cytokines in tumor invasion and angiogenesis. In Goldberg ID, Rosen EM (eds): "Hepatocyte Growth Factor-Scatter Factor (HGF-SF) and the c-*Met* Receptor." Basel: Birkhäuser Verlag, pp 301–310.

Santos OFP, Nigam SK (1993): HGF-induced tubulogenesis and branching of epithelial cells is modulated by extracellular matrix and TGF-β. Dev Biol 160:293–302.

Schnaper HW, Grant DS, Stetler-Stevenson WG, Fridman R, D'Orazi G, Murphy AN, Bird RE, Hoythya M, Fuerst TR, French DL, Quigley JP, Kleinman HK (1993): Type IV collagenase(s) and TIMPs modulate endothelial morphogenesis in vitro. J Cell Physiol 156:235–246.

Shaughnessy SG, Whaley M, Lafrenie RM, Orr FW (1993): Walker 256 tumor cell degredation of extracellular matrices involves a latent gelatinase activated by reactive oxygen species. Arch Biochem Biophys 304:314–321.

Stein CA (1993): Suramin: A novel antineoplasmic agent with multiple potential mechanisms of actin. Cancer Res 53:2239–2248.

Takaki S, Kuratsu J, Minhara Y, Yamada M, Ushio Y (1992): Endothelial cell chemotactic factor derived from human glioma cell lines. J Neurosurg 76:822–829.

Tanimoto H, Yoshida K, Yokozaki H, Yasui W, Nakayama H, Ito H, Ohama K, Tahara E (1991): Expression of basic fibroblast growth factor in human gastric carcinomas. Virchows Arch B Cell Pathol 61:263–267.

Trojan J, Johnson TR, Rudin SD, Ilan J, Tykocinski ML, Ilan J (1993): Treatment and prevention of rat glioblastoma by immunogenic C6 cells expressing antisense insulin-like growth factor I RNA. Science 259:94–97.

Vernon RB, Angello JC, Iruela-Arispe ML, Lane TF, Sage EH (1992): Reorganization of basement membrane matrices by cellular traction promotes the formation of cellular networks in vitro. Lab Invest 66:536–547.

Vukicevic S, Kleinman HK, Luyten FP, Roberts AB, Roche NS, Reddi AH (1992): Identification of multiple active growth factors in basement membrane Matrigel suggests caution in interpretation of cellular activity related to extracellular matrix components. Exp Cell Res 202:1–8.

Wade TP, Kasid AK, Stein CA, LaRocca RV, Sargent ER, Gomella LG, Myers CE, Linehan WM (1992): Suramin interference with transforming growth factor-β inhibition of human renal cell carcinoma in culture. J Surg Res 53:195–198.

Westermann R, Unsicker K (1990): Basic fibroblast growth factor (bFGF) and rat C6 glioma cells: Regulation of expression, absence of release, and response to exogenous bFGF. Glia 3:510–521.

Yang EY, Moses HL (1990): Transforming growth factor β-1 induces changes in cell migration, proliferation, and angiogenesis in the chick chorioallantoic membrane. J Cell Biol 111:731–741.

Zugmaier G, Lippman ME, Wellstein A (1992): Inhibition by pentosan polysulfate (PPS) of heparin-binding growth factors released from tumor cells and blockage by PPS of tumor growth in animals. J Natl Cancer Inst 84:1716–1724.