Cytochalasin E, an Epoxide Containing Aspergillus-Derived Fungal Metabolite, Inhibits Angiogenesis and Tumor Growth

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ABSTRACT

Several previously identified inhibitors of angiogenesis have been epoxide-containing fungus-derived metabolites. We therefore hypothesized that novel epoxide-containing low molecular weight compounds structurally resembling known antiangiogenic agents may also exhibit antiangiogenic activity. Cytochalasin E was found to be a potent and selective inhibitor of bovine capillary endothelial (BCE) cell proliferation. Cytochalasin E differed from other cytochalasins by the presence of an epoxide. The epoxide was required for activity, because acid-catalyzed hydrolysis of the epoxide abrogated the specificity and potency of cytochalasin E. Phalloidin staining indicated that disruption of actin stress fibers by cytochalasin E occurred only at relatively high concentrations. Lower concentrations of cytochalasin E preferentially inhibited BCE cell proliferation without disrupting actin stress fibers. In vivo, cytochalasin E inhibited angiogenesis induced by basic fibroblast growth factor by 40% to 50% in the mouse cornea assay and inhibited the growth of Lewis lung tumors by approximately 72%. Cytochalasin E is a potent antiangiogenic agent that may hold promise for the treatment of cancer and other types of pathologic angiogenesis.

During angiogenesis, a gradient of growth factors induce sprouting from vessels by stimulating proliferation and migration of endothelial cells (Folkman and Klagsbrun, 1987). Agents that inhibit the migration or the proliferation of endothelial cells may potentially be used as treatments for angiogenesis-dependent diseases such as cancer, diabetic retinopathy, and arthritis (Folkman, 1995a,b). In addition, therapy that specifically targets endothelial cells should have fewer side effects than cytotoxic chemotherapy, which targets tumor cells but also affects normal cells due to a lack of selectivity (Folkman, 1995b).

For these reasons, there has been significant interest in the discovery and the identification of low molecular weight inhibitors of angiogenesis. A number of reported low molecular weight inhibitors of angiogenesis are fungus- or microbe-derived metabolites containing epoxides (Ingber et al., 1990; Oikawa et al., 1991, 1995; Onozawa et al., 1997). The cytochalasins are a family of compounds with diverse activities on cellular function, including inhibition of actin polymerization and glucose transport (Carter, 1967; Buchi et al., 1973; Brenner and Korn, 1980; Mookerjee et al., 1981). We found that cytochalasin E, an epoxide-containing metabolite of Aspergillus clavatus, contains a substructure spanning an epoxide group found in TNP-470 (AGM-1470) (Ingber et al., 1990). TNP-470, an Aspergillus-derived angiogenesis inhibitor, is currently in phase III trials for the treatment of cancer. Due to structural similarities between cytochalasin E and TNP-470, we hypothesized that cytochalasin E may exhibit antiangiogenic activity. We found that cytochalasin E was a particularly potent and selective inhibitor of endothelial cells in vitro and that it inhibited angiogenesis and tumor growth in vivo. Unlike TNP-470, however, cytochalasin E did not inactivate methionyl aminopeptidase-2 (Griffith et al., 1997; Sin et al., 1997). Thus, cytochalasin E is a novel inhibitor of angiogenesis and tumor growth, which may be useful in the treatment of cancer and other types of angiogenesis-dependent diseases.

Materials and Methods

Reagents and Cell Culture. Cytochalasins E, D, H, and A were purchased from Aldrich Chemical Co. (Milwaukee, WI). The cytochalasins are a family of compounds with diverse activities on cellular function, including inhibition of actin polymerization and glucose transport (Carter, 1967; Buchi et al., 1973; Brenner and Korn, 1980; Mookerjee et al., 1981). We found that cytochalasin E, an epoxide-containing metabolite of Aspergillus clavatus, contains a substructure spanning an epoxide group found in TNP-470 (AGM-1470) (Ingber et al., 1990). TNP-470, an Aspergillus-derived angiogenesis inhibitor, is currently in phase III trials for the treatment of cancer. Due to structural similarities between cytochalasin E and TNP-470, we hypothesized that cytochalasin E may exhibit antiangiogenic activity. We found that cytochalasin E was a particularly potent and selective inhibitor of endothelial cells in vitro and that it inhibited angiogenesis and tumor growth in vivo. Unlike TNP-470, however, cytochalasin E did not inactivate methionyl aminopeptidase-2 (Griffith et al., 1997; Sin et al., 1997). Thus, cytochalasin E is a novel inhibitor of angiogenesis and tumor growth, which may be useful in the treatment of cancer and other types of angiogenesis-dependent diseases.
lasin E derivative JHS-2-35 was formed by bubbling HCl gas in a solution of cytochalasin E dissolved in chloroform for 1 h. The product was confirmed by silica gel HPLC as well as by NMR (Kajimoto et al., 1989). The ozonolysis product of cytochalasin E was synthesized as described (Aldridge et al., 1973). Primary bovine capillary endothelial (BCE) cell cultures (Folkman et al., 1979) were plated in plastic tissue culture wells pretreated with 1.5% gelatin/PBS for 30 min at room temperature. The cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% bovine calf serum, 1 ng/ml basic fibroblast growth factor (bFGF), 2 mM L-glutamine, 1 mM sodium pyruvate, and 100 U/ml penicillin-streptomycin in an atmosphere of 10% CO2, NIH-3T3 [American Type Culture Collection (ATCC), Manassas, VA], the ST7 human gastric carcinoma (Yadav et al., 1994), A375 (ATCC), and MMAN (obtained from Dr. J. Arbiser, Emory University, Atlanta, GA) melanomas, retinal pigment epithelial cells (obtained from Dr. A. Adams, Children’s Hospital, Boston, MA), bovine smooth muscle cells (Dr. P. D’Amore, Children’s Hospital), and C6 glioblastoma (ATCC), were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, and 100 U/ml penicillin-streptomycin. The low metastatic variant of the Lewis lung carcinoma line (obtained from M. S. O’Reilly, Children’s Hospital) was maintained in C57BL/6 mice as described (O’Reilly et al., 1994).

**Proliferation Assay.** To determine proliferation, 4000 cells per well were plated in 200 μl of the appropriate media in 96-well tissue culture plates together with drugs in varying concentrations. The final dimethyl sulfoxide (DMSO)vehicle concentration did not exceed 0.1%. The cells were plated in a 37°C, humidified incubator containing 10% CO2. After 2 days, the cells were stained with methylene blue according to the method of Goldman and Bar-Shavit (1979). Briefly, the plates were inverted to remove media, the wells were washed once with PBS, and then the cells were fixed with 4% paraformaldehyde in PBS containing Ca2+ and Mg2+ for 20 min at room temperature. The cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, and 100 U/ml penicillin-streptomycin. The low metastatic variant of the Lewis lung carcinoma line (obtained from M. S. O’Reilly, Children’s Hospital) was maintained in C57BL/6 mice as described (O’Reilly et al., 1994).

**Phalloidin Staining.** BCE cells (20,000 cells/well) were plated on gelatinized circular coverslips in 24-well tissue culture dishes and allowed to attach overnight. After treatment with cytochalasin E, the cells were washed once with PBS and then fixed with 4% paraformaldehyde in PBS containing Ca2+ (1.71 mM final) and Mg2+ (0.93 mM final) for 30 min at room temperature. The fixed cells were washed once with PBS and then permeabilized for 20 min at room temperature in PBS containing 0.5% Triton X-100, 1.71 mM Ca2+, 0.93 mM Mg2+, and 0.5% bovine serum albumin. The permeabilized cells were incubated with 1 μM tetramethylrhodamine isothiocyanate (TRITC)-phalloidin (Sigma) dissolved in permeabilization buffer (diluted from a 100 μM stock in DMSO) for 60 min at 37°C. The labeled cells were washed twice with PBS containing Ca2+ and Mg2+ and then mounted onto slides with Fluoromount-G (Southern Biotechnology Associates, Inc., Birmingham, AL).

**Mouse Corneal Neovascularization.** The mouse cornea model for neovascularization was performed as previously described (Kenyon et al., 1996). Briefly, a corneal pocket was created in the eyes of 7- to 9-week-old C57BL/6 mice, and a 0.4-× 0.4-mm, sucrate-sulfate (Sigma) pellet containing 50 to 100 ng of bFGF coated with hydroxypropyl was implanted in the micropocket. The pellet was positioned 0.7 to 1.0 mm from the temporal limbus, and erythrocin was applied once to the operated eye. A 10 mg/ml stock solution of drugs dissolved in DMSO was stored in aliquots at −20°C. On the day of injection, the drugs were thawed, diluted to 0.5 mg/ml in olive oil, vortexed for 5 min at room temperature, and then administered s.c. starting on the day of implantation. For each treatment group, at least seven eyes were quantitated and repeated at least one time. Five days after pellet implantation, the maximal vessel length and number of clock hours of neovascularization was measured using a slit lamp biomicroscope as described previously (Kenyon et al., 1996). The area of corneal neovascularization was calculated according to a modified formula for a half-ellipse: Area (mm2) = [(π × clock hours × length (mm)) × 0.2 mm].

**In Vivo Tumor Growth.** The Lewis Lung tumor line was maintained by in vivo passage as described (O’Reilly et al., 1994). In brief, C57BL/6 mice bearing Lewis lung tumors of 600- to 1200-mm3 volume were sacrificed, and the tumors were resected under aseptic conditions. A suspension of the resected tumor cells was made by passage through a sieve in 0.9% normal saline and then sequentially through 22- to 30-gauge needles. Approximately 1 million tumor cells in 0.1 ml of saline were injected s.c. in the dorsa of mice (weighing approximately 25 g) in the proximal midline. Starting 5 days after tumor implantation, the mice were injected with drugs s.c. away from the tumor near the flank using at least a 26-gauge needle. Each treatment group consisted of at least four mice, and each experiment was repeated. Tumor measurements were made using a caliper, and the volumes were calculated according to the formula: tumor volume = (width)2 × length × 0.52.

**Methionin Aminopeptidase Assay.** Varying amounts of the inhibitors were incubated with 10 nM purified human methionyl aminopeptidase-2 (MetAP-2) in buffer H [containing 10 mM HEPES, pH 7.35, 100 mM KCl, 10% glycerol, and 0.1 mM Co(II)] and incubated at 37°C for 15 min. To start the enzyme reaction, 1 mM Met-Oly-Met-Met was added to the reaction mixture. Released methionine was quantified at 0, 3, and 5 min using the method of Zuo et al. (1994).

**Results**

**Cytochalasin E Shows a Unique Inhibition of BCE Cells.** We investigated the effect of cytochalasin E and related molecules (Fig. 1, compounds 1, 4–8) on the proliferation of capillary endothelial cells as well as other nonendothelial cell lines. In Table 1, the sensitivity of BCE cells were compared with several different cell lines, including primary smooth muscle and retinal pigment epithelial cells, the NIH-3T3 fibroblast line, and the tumor lines ST7, A375, and C6. Among these cell lines, the BCE cells were the most sensitive to inhibition by cytochalasin E, particularly at lower concentrations. We then compared the inhibition of BCE proliferation by cytochalasins E, A, and H and found that, among these compounds, cytochalasin E was the most potent (Fig. 2). Cytochalasins A and H inhibited BCE cell proliferation at nanomolar concentrations, whereas cytochalasin E inhibited BCE proliferation at low picomolar concentrations.

The inhibition of BCE cell proliferation by cytochalasin E was biphasic and occurred over a broad concentration range (Fig. 2). The first phase of inhibition occurred in the femtomolar to picomolar concentrations. At these dose levels, there was no visible cytotoxic effect (not shown). The cells were able to proliferate, because cell numbers increased after 2 days, but proliferation was significantly reduced. The second phase occurred in the nanomolar to micromolar range, which corresponded roughly to the range of inhibition seen using other cytochalasins. In this range, there were gross changes in cell morphology and loss of adhesion to the substratum. At these higher concentrations, the observed inhibition was due in part to cell death (as determined by trypan blue staining) and to loss of adhesion to substratum. The biphasic nature of
the inhibition and the associated differences in morphology suggested that cytochalasin E inhibited proliferation through more than one site of action. At high concentrations (10^{-9} to 10^{-6} M), cytochalasin E, as well as cytochalasins A and H, inhibited proliferation predominantly through its well known antiactin effect (Brenner and Korn, 1980; Flanagan and Lin, 1980). Inhibition at lower concentrations (10^{-14} to 10^{-9} M), however, was mediated by an interaction with a different target.

In contrast to BCE cells, cytochalasin E inhibited NIH-3T3 fibroblasts only in the nanomolar to micromolar range, whereas inhibition at lower concentrations of cytochalasin E was not observed. In fact, the fibroblast line was equally sensitive to inhibition by cytochalasins E, A, and H as shown

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**Fig. 1.** Structure of fungus-derived metabolites and angiogenesis inhibitors. Cytochalasin E (1); TNP-470 (2); fumagillin (3); and cytochalasins A (4), D (5), and H (6). Rearrangement product JHS-2-35 (7) and ozonolysis product JHS-1-99 (8) of cytochalasin E. Regions of similarity containing epoxides are indicated by the bold lines.
by roughly overlapping dose responses (Fig. 2). Other cell types, including primary smooth muscle cells, tumor lines, and retinal pigment epithelial cells, similar to NIH-3T3 fibroblasts, were much less sensitive to cytochalasin E (Table 1). The inhibitory concentrations for NIH-3T3 fibroblasts by cytochalasins approximately corresponded to the second phase (10^{-9} to 10^{-6} M) of BCE cell inhibition. Therefore, at the lower concentrations, cytochalasin E inhibited BCE cell proliferation through a unique interaction, which was not observed in other cell types. At the higher concentrations (10^{-9} to 10^{-6} M), cytochalasin E exhibited a cytochalasin-like effect characterized by disruption of actin leading to growth inhibition and cytotoxicity of various cells types.

BCE cells were treated with varying concentrations of cytochalasin E for 16 h and then stained with phalloidin to visualize the effect of cytochalasin E on actin (Wulf et al., 1979). At 2 pM, dissolution of actin filament was not evident compared with controls (Fig. 3), even though the proliferation of BCE cells was inhibited at this concentration (Fig. 2). Dissolution of actin stress fibers was not seen until much higher concentrations of cytochalasin E were used. At 20 nM and higher, an antiactin effect was revealed by the absence of stress fiber staining, retraction of the cytoplasm, and cellular detachment from the substratum.

Because the epoxide group was one of the distinguishing structural elements of cytochalasin E, the contribution of the epoxide was examined by acid-catalyzed rearrangement of the epoxide group under nonaqueous conditions. This resulted in a single rearrangement product, JHS-2-35 (Fig. 1, compound 7), whose perisohydroindole core ring was now identical with other cytochalasins. JHS-2-35 exhibited a sharper inhibitory dose response, which was evidence for a more restricted mechanism of action. The rearrangement product was no longer inhibitory for BCE proliferation at picomolar concentrations but was still active at the higher doses (Fig. 4). The inhibition at the higher doses was associated with cytoplasmic contraction and cell rounding (not shown), which demonstrated that JHS-2-35 was still able to inhibit actin polymerization. The similar dose-response profile of JHS-2-35 and cytochalasin A revealed that the two compounds were active at similar concentrations. Therefore, the epoxide of cytochalasin E participated in the unique inhibition of BCE cell proliferation but, as might be expected, was not required for inhibition of cellular proliferation caused by dissolution of actin. Cleavage of the macrocyclic ring by ozonolysis resulted in compound JHS-1-99 (Fig. 1, compound 8), which was inactive in the proliferation assays (Fig. 4). This suggests that elements of the macrocyclic ring participate in both the selective inhibition of BCE proliferation as well as nonselective inhibition of actin polymerization.

Inhibition of Angiogenesis by Cytochalasin E in an Experimental Eye Model. To determine whether cytochalasin E exhibited antiangiogenic activity, cytochalasin E was tested in vivo in a mouse cornea angiogenesis model (Kenyon et al., 1996). In Fig. 5, neovascularization was induced by bFGF released slowly from a polymer implanted in the mouse cornea. Starting on the day of surgery, the animals were treated with doses of cytochalasin E based on previous animal studies (Trirawatanapong et al., 1980). Cytochalasin E was mixed and diluted in olive oil to retard its release and then administered s.c. Delivery by oral or i.p. routes resulted in reduced effectiveness. At a maximum tolerated dose of 2.5 mg/kg every other day, cytochalasin E inhibited bFGF-induced angiogenesis by approximately 50% (Fig. 5) without evidence of toxicity. Vascular endothelial growth factor (VEGF)-induced angiogenesis was also equally inhibited (not shown).

Table 2 shows a comparison of cytochalasin E with other cytochalasins. At 2.5 mg/kg/day, cytochalasin E inhibited bFGF-induced angiogenesis by 50%, but at this dose, cytochalasins A and H were both toxic and resulted in significant weight loss (>5% of body weight). At lower doses, cytochalasins A and H also exhibited some antiangiogenic activity. At comparable doses, however, cytochalasin E was the most effective (Table 2). At 2.5 mg/kg every other day, cytochalasin E inhibited 39 ± 3%, whereas cytochalasin A and cytochalasin H inhibited 26 ± 4% (P < .02) and 22 ± 3% (P < .001), respectively. At 2.0 mg/kg every other day, cytochalasin E inhibited angiogenesis by 36 ± 2%, whereas cytochalasin A and cytochalasin H inhibited 22 ± 2% (P < .002) and 27 ± 4% (P < .04), respectively. Thus, cytochalasin E was less toxic and significantly more potent than either cytochalasin A or H.

Inhibition of Tumor Growth in Vivo by Cytochalasin E. Because cytochalasin E inhibited angiogenesis induced by bFGF and VEGF in mice, cytochalasin E was also tested for inhibiting the growth of the Lewis lung tumor in mice. Mice were treated over a 2-week period at the maximum tolerated dose of 2.5 mg/kg every other day starting 5 days after inoculation of tumor cells. Tumor volumes and animal weights were monitored throughout the course of treatment.

As shown in Fig. 6, cytochalasin E inhibited the growth of Lewis lung tumors with a final T/C (tumor volume of treated animals/tumor volume of control animals) of 0.28 (72% inhibition). Table 3 summarizes the effect of cytochalasins at varying doses. At 1.0 mg/kg/day, cytochalasin E inhibited tumor growth with a final T/C value of approximately 0.77 (23% inhibition). A dose of 2.0 mg/kg every 3 days gave approximately the same degree of inhibition (T/C = 0.7). Increasing the dose to 2.0 mg/kg administered every other day resulted in a final T/C value of 0.28 (72% inhibition). Higher doses resulted in weight loss. At the maximum tolerated dose, cytochalasin A and cytochalasin H were much less effective with final T/C values of 0.59 (41% inhibition), and 0.65 (35% inhibition), respectively. These results showed...
that, among the cytochalasins, cytochalasin E was the most effective inhibitor of tumor growth as well as angiogenesis.

**Cytochalasin E Does Not Inhibit MetAP-2.** Based on structural resemblance of cytochalasin E to TNP-470 (Fig. 1), a previously identified inhibitor of endothelial proliferation and angiogenesis (Ingber et al., 1990), we investigated the possibility that cytochalasin E and TNP-470 might interact with a common target. The angiogenesis inhibitors fumagillin and TNP-470 are known to bind and to inactivate the enzyme MetAP-2 (Griffith et al., 1997; Sin et al., 1997). To
determine whether inhibition of MetAP-2 could also be involved in nonactin-mediated inhibition of BCE cells, the peptidase activity of recombinant human MetAP-2 on a synthetic peptide substrate was measured in the presence of increasing concentrations of cytochalasin E and TNP-470. Approximately 20 nM TNP-470 completely inhibited the activity of 10 nM MetAP-2 as measured by release of methionine using a synthetic peptide substrate at 1 mM. In contrast, cytochalasin E was unable to inhibit MetAP-2 activity even when tested at a 100-fold higher concentration of TNP-470 used to completely suppress MetAP-2 (not shown). Therefore, despite the similarity in structure, the mechanism of cytochalasin E-mediated suppression of endothelial proliferation and angiogenesis appeared distinct from that of TNP-470.

Discussion

The cytochalasins are a class of fungus-derived metabolites whose effects on cellular function include inhibition of glucose transport, inhibition of actin polymerization (Brenner and Korn, 1980; Flanagan and Lin, 1980), blockage of cytoplasmic cleavage, and inhibition of cell movement (Carter, 1967). Cytochalasin E is an epoxide-containing cytochalasin family member, which was isolated as a minor metabolite of the food storage mold

Structurally, the cytochalasins are a family of compounds (Fig. 1) characterized by a central perisohydroindole core ring and an attached large macrocyclic ring, which varies in

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**TABLE 2**

Inhibition of angiogenesis in the mouse cornea by cytochalasins

Drugs were dissolved in DMSO as a stock solution, and then administered S.C. in oil. Treatment began on the day of surgery. Angiogenesis in the mouse cornea induced by bFGF was determined on day 5. Neovascularization was quantitated, and the percentage inhibition was determined by the formula described under Material and Methods.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>% Inhibition (±S.E.)</th>
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<tbody>
<tr>
<td></td>
<td>Cytochalasin E</td>
</tr>
<tr>
<td>2.5 qd*</td>
<td>50 ± 3</td>
</tr>
<tr>
<td>2.0 qd</td>
<td>49 ± 4</td>
</tr>
<tr>
<td>2.5 qod</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>2.0 qod</td>
<td>36 ± 2</td>
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* qd, everyday; qod, every other day.

a ND, not determined due to toxicity.

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**TABLE 3**

Inhibition of tumor growth by cytochalasins

Lewis lung tumors were injected in the dorsa of C57BL/6 mice. Beginning 5 days after injecting tumors, cytochalasin E was diluted in olive oil from a stock solution of cytochalasin E at 10 mg/ml dissolved in DMSO and administered s.c. as indicated. Tumor volumes were calculated according to the formula described under Material and Methods.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>T/C</th>
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<tbody>
<tr>
<td>Cytochalasin E 2.0 mg/kg qod</td>
<td>0.28</td>
</tr>
<tr>
<td>2.0 mg/kg q3d</td>
<td>0.70</td>
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<tr>
<td>1.0 mg/kg qd</td>
<td>0.77</td>
</tr>
<tr>
<td>Cytochalasin A 2.5 mg/kg qod</td>
<td>0.59</td>
</tr>
<tr>
<td>Cytochalasin H 2.5 mg/kg qod</td>
<td>0.65</td>
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</table>

* q3d, every third day.

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E-mediated suppression of endothelial proliferation and angiogenesis appeared distinct from that of TNP-470.

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**Fig. 5**. Inhibition of bFGF-induced angiogenesis in the mouse cornea by cytochalasin E. Mice with corneal implants of polymers containing bFGF were treated starting on the day of implantation with cytochalasin E injected s.c. at a dose of 0 (control) or 2.5 mg/kg every other day. 5 days after pellet implantation, angiogenesis in the cornea was visualized by slit lamp biomicroscopy.

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**Fig. 6**. Inhibition of tumor growth by cytochalasin E. Lewis lung tumors were implanted in C57BL/6 mice. On the 5th day after tumor injection, the mice were treated with either cytochalasin E administered at 2 mg/kg every other day for 15 days (●) or left untreated (○). Tumor volumes were calculated as described under Materials and Methods.
size and composition. A carbonyl group is present at carbon 1 of the central core ring, and a methyl group is present on carbon 5. Most cytochalasins contain a hydroxyl group on carbon 7, but cytochalasin E (Fig. 1, compound 1), instead, has a 6,7-epoxy group.

A broad, biphasic inhibitory range observed in vitro suggested the existence of at least two distinct targets. Loss of the epoxide by rearrangement resulted in a product that was no longer inhibitory for BCE cell proliferation at picomolar concentrations. At higher doses, however, the rearrangement product was still inhibitory and induced morphologic changes consistent with disruption of actin. Thus, cytochalasin E inhibited proliferation by disruption of actin at higher concentrations and also by an epoxide-dependent mechanism at lower concentrations. In vivo, inhibition of angiogenesis by several different cytochalasins suggested that disruption of actin could contribute to inhibition of angiogenesis. The greater potency of cytochalasin E, however, appeared to be attributable to an interaction between cytochalasin E and an as yet unidentified molecule involved in preferential inhibition of endothelial cells.

Interestingly, cytochalasin E contains an arrangement of atoms that span elements of the macrocyclic ring, the epoxide group, and the core ring found in the conserved regions of the angiogenesis inhibitor TNP-470 and its parent compound (Fig. 1, compounds 1 to 3). TNP-470 and fumagillin were similar over a greater portion of the molecule to cytochalasin E than to the other cytochalasins. In addition, cytochalasin E is a fungus-derived metabolite of Aspergillus (Aldridge et al., 1973), which exhibited potent and selective inhibition of endothelial cell proliferation similar to that of fumagillin and TNP-470 (Ingber et al., 1990). At high concentrations, fumagillin, the parent compound of TNP-470, exhibited cell-rounding activity resembling a cytochalasin-like effect (Ingber et al., 1990). Nevertheless, whereas TNP-470 in vitro completely suppressed the activity of MetAP-2 (Griffith et al., 1997; Sin et al., 1997), cytochalasin E had no effect. Thus, cytochalasin E appeared to specifically inhibit endothelial cell proliferation and angiogenesis by a mechanism distinct from that of TNP-470.

Although cytochalasin E is inhibitory for capillary cells in the picomolar range, plasma from mice injected with cytochalasin E exhibited little inhibitory effect on BCE proliferation (not shown). This suggests that cytochalasin E may be metabolized, bound to tissue, or rapidly cleared from circulation. The epoxide of cytochalasin E appears to be necessary for activity, and studies with TNP-470 have shown that the epoxide is cleaved rapidly in circulation (Figg et al., 1997). This may also explain why both cytochalasin E and TNP-470 are active at picomolar concentrations in culture but require milligram per kilogram doses in vivo.

Current efforts are directed toward the development of cytochalasin E analogs lacking antiactin activity, which may allow higher administration of the drug. The identification of the nonactin target of cytochalasin E would facilitate the development of more specific analogs and may reveal new signaling pathways involved in angiogenesis and vascular development. Finally, we propose that cytochalasin E and analogs may be useful for the treatment of angiogenesis-dependent diseases such as cancer and age-related macular degeneration.

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