Cancer Chemother Pharmacol (1999) 44: 411–416

ORIGINAL ARTICLE

Mark K. Clements · Carleton B. Jones
Melinda Cumming · Sayed S. Daoud

Antiangiogenic potential of camptothecin and topotecan

Received: 1 October 1998 / Accepted: 8 March 1999

Abstract *Purpose*: To determine the inhibitory nature of sublethal doses of camptothecin (CPT) and topotecan (TPT) treatments on normal human endothelial cells in vitro, as well as the in vivo antiangiogenic activity as compared to another antiangiogenic compound, TNP-470 and to a nonspecific cytotoxic agent, cisplatin. *Methods*: Growth inhibition was determined by the crystal violet assay to measure relative cell numbers. ^{3}H-thymidine uptake was used to determine the inhibitory effect of CPT and TPT on DNA synthesis in vitro. Cell viability was determined using trypan blue exclusion assays. Cell cycle response to CPT was determined by flow cytometric analysis of propidium iodide-stained nuclei. In vivo inhibition of angiogenesis was determined by the disc angiogenesis system (DAS), where surgical sponge discs were placed subcutaneously in the rat dorsum and the ability of systemic treatment with liposomal CPT (LCPT), TPT, TNP-470 or cisplatin to inhibit vascular growth into the discs was evaluated. Quantitation of vascular growth was determined using toluidine blue staining of sectioned discs followed by digital image analysis. *Results*: Treatment with 50 n*M* CPT or TPT inhibited human umbilical venular endothelial cell (HUVEC) growth as shown by crystal violet staining, but was not cytotoxic to the cells. This was evidenced by the fact that cell numbers did not increase or decrease with treatment, but remained static while cells were viable for over 96 h posttreatment. ^{3}H-thymidine uptake in HUVEC was inhibited as early as 5 min, reached a maximum inhibition at 24 h and lasted over 96 h posttreatment. Cell cycle analysis of CPT-treated HUVEC showed arrest in S-phase at 12 h with a concurrent decrease in population of cells in G_1. Accumulation of cells at the G_2/M-phase was discernible at 24 h along with the S-phase inhibition. Treatment of rats with 1 mg/kg LCPT or TPT every other day for 14 days resulted in approximately 30% inhibition of vascular growth into the discs. This inhibition was similar to the inhibition seen with TNP-470, an established and potent angiogenic inhibitor. In contrast, cisplatin was not as effective in inhibiting vascular growth into the discs. *Conclusions*: In this work we showed that CPT and TPT inhibit human endothelial cell growth in vitro in a non-cytotoxic manner and that this inhibition lasts more than 96 h after drug removal. We also showed that LCPT and TPT, unlike a nonspecific cytotoxic agent, cisplatin, are as effective as TNP-470 in inhibiting angiogenic growth in the in vivo disc angiogenesis model. From this observation we propose that in addition to their proven tumoricidal activities, camptothecins may have an indirect in vivo antitumor effect mediated through the inhibition of angiogenesis.

Key words Camptothecin · Topotecan · TNP-470 · Angiogenesis · Endothelial cells · Differentiation

Supported in part by Public Service Grant CA67265 from the National Cancer Institute and Susan G. Komen Foundation for Breast Cancer Portland Chapter to SSD. This work is part of the doctoral thesis of M.C.

M.K. Clements[1] · C.B. Jones · S.S. Daoud
Pharmacology and Toxicology Graduate Program,
College of Pharmacy, Washington State University,
Pullman, WA 99164, USA

S.S. Daoud (✉)
Department of Pharmaceutical Sciences,
College of Pharmacy, Washington State University,
Pullman, WA 99164, USA
E-mail: daoud@mail.wsu.edu
Tel.: +1-509-335-8910; Fax: +1-509-335-0162

M. Cumming
V.C.A.P. Department, Washington State University, Pullman,
WA 99164, USA

Present address:
[1] Montana State University,
Department of Veterinary Molecular Biology,
Bozeman, MT 59717, USA

Introduction

Classic antineoplastic chemotherapy has focused on directly killing or inhibiting the growth of tumor cells.

This approach, however, typically affects normal cells in addition to tumor cells. More recently, innovative strategies with greater selectivity for tumor versus host tissues have been developed, with targets such as oncogenes and elements of cell signaling pathways. Another potential target is being realized based on the finding that tumors are dependent on a switch to an angiogenic phenotype and the subsequent formation of new vasculature [14, 17]. Thus, inhibition of tumor blood supply has been realized as a unique approach to stop tumor growth [11, 18], and hence research has focused on the discovery of antiangiogenic agents [13]. Two of these agents that have been shown to selectively inhibit endothelial cell proliferation in vitro and tumor growth in vivo are TNP-470 [40] and pentosan sulfate [39]. More recently, other agents such as angiostatin, endostatin, linomide, laminarin sulfate, thalidomide and 2-methoxy-estradiol have also been found to inhibit the process of angiogenesis [7, 16, 20, 32, 38].

The increasing focus on antiangiogenic therapy has led to the rational search for agents with selective effects on endothelial cell proliferation with the assumption that a high proliferation rate of endothelial cells within tumor tissue will permit improved selectivity against tumor versus normal tissues [14]. This "angiosuppressive" effect could possibly be accomplished by using known chemotherapeutic agents such as ET-18-OCH_3, paclitaxel, flavone acetic acid, bleomycin, methotrexate and mitoxantrone [4, 25, 34], suggesting that a hidden "antivascular" effect may be operating in a number of conventional anticancer therapies [35]. More recent work in our laboratory has revealed that camptothecin (CPT), a topoisomerase I inhibitor, possesses in vitro cytotoxic activity against tumor cells and antiproliferative activity against endothelial cells [5, 23]. We have also reported that CPT has effective antitumor activity in mice xenografted with human breast carcinoma [9]. However, the reported experimental results do not allow conclusions concerning the extent to which potential antivascular effects contribute to the in vivo antitumor effect of CPT.

In the study reported here, we showed that CPT and its analog topotecan (TPT) inhibited the growth of human umbilical venular endothelial cells (HUVECs) at drug concentrations that were not cytotoxic to these normal human endothelial cells. We also showed that this in vitro inhibitory effect was translated into an inhibition of angiogenesis in the disc angiogenesis model (DAS) in vivo. The results from these experiments suggest that CPT and its analogs may inhibit tumor growth possibly by inhibiting tumor-induced angiogenesis as well as by a direct cytotoxic activity against tumor cells.

Materials and methods

Chemicals and supplies

CPT (NSC 94600) and TPT (NSC 609699) were obtained from the Drug Development Branch, National Cancer Institute, NIH (Bethesda, Md.). They were dissolved in DMSO at 4 m*M*, aliquoted and stored at −20 °C. Further dilutions were made in DMSO just before use. The final concentration of DMSO in culture did not exceed 0.1% (v/v) which is nontoxic to cells. TNP-470 (AGM-1470) was a gift from Dr. Deborah Milkowski, TAP Pharmaceuticals (Deerfield, Ill.). Cholesterol (CHOL) and vascular endothelial growth factor (VEGF) were purchased from Sigma Chemical Co. (St. Louis, Mo.), basic fibroblast growth factor (bFGF) was obtained from Gibco BRL (Bethesda, Md.), and cisplatin (CDDP; Platinol) was obtained from Bristol Myers Squibb Laboratories (Syracuse, N.Y.).

Dipalmitoylphosphatidylcholine (DPPC), phosphatidylinositol (PI) and sphingomyelin were purchased from Avanti Polar Lipids (Birmingham, Ala.). [Methyl-^{3}H]-thymidine (64 Ci/mmol) was purchased from Andotech Life Sciences (Irvine, Calif.). Surgical sponge was purchased from Shima America Corporation (Carol Stream, Ill.). All other chemicals were reagent grade.

Cell culture

HUVEC were purchased from Clonetics (San Diego, Calif.) and maintained in endothelial growth medium, EndoPack-UV, supplied by Clonetics, in a humidified incubator at 37 °C in an atmosphere containing 5% CO_2. Cells were passaged when subconfluent, and split 1:4. Cells were examined by phase contrast microscopy daily and cultures were used within the first ten passages.

Growth inhibition and antiproliferative assays

HUVECs were plated into 24-well tissue culture plates at 2×10^4 cells/well and grown under standard culture conditions with an additional 30 ng/ml each of bFGF and VEGF. Following 24 h treatment with 50 n*M* of CPT, 50 n*M* TPT or vehicle, cells were washed and incubated in fresh medium. Relative cell numbers were then determined using the crystal violet staining assay at 0, 24, 36, 48, 72 and 96 h following treatment.

The antiproliferative activities of CPT and TPT were measured by the loss of ^{3}H-thymidine incorporation into cellular DNA as described previously [17, 18]. Cells were plated and treated with 50 n*M* CPT, 50 n*M* TPT, or vehicle. Samples were processed at the same time-points as indicated in the crystal violet assay, rinsed twice with phosphate-buffered saline (PBS, pH 7.4) and radiolabeled with 1 mCi/ml ^{3}H-thymidine for 4 h under standard culture conditions. The cells were then fixed with methanol/acetic acid 3:1 for 2 h, rinsed twice with 80% methanol and finally digested for 1 h with 0.1 *M* NaOH. The radioactivity of each sample was measured in a TRI-CARB 2500 TR liquid scintillation analyzer. The studies were carried out on quadruplicate samples and the results are expressed as the percentages relative to the control counts.

Liposome preparation

Multilamellar liposome-incorporated camptothecin (LCPT) for in vivo angiogenesis study was prepared as previously described [5, 23].

Cell cycle analysis and flow cytometry

Cells in 100-mm tissue culture dishes were treated with 50 n*M* CPT or vehicle for 24 h. At selected time-points, cells were washed with cold PBS, pH 7.4, harvested by centrifugation and incubated on ice in 70% ethanol. Following fixation, cells were washed twice with ice-cold PBS and incubated overnight at 4 °C in PBS containing 50 μg/ml PI, 100 μg/ml RNase A and 0.1% Triton X-100. Samples were analyzed with a FACScan flow cytometer (Becton Dickinson), and the fractions of cells in G_0/G_1, S and G_2/M phases were calculated using the software provided by the manufacturer.

In vivo angiogenesis

A modification of the DAS was used for the quantitation of angiogenesis in an in vivo rat model [28, 31]. Discs were constructed such that an angiogenic factor embedded in a polymer and placed in the center of the disc could diffuse out and induce vascular growth within the disc. The discs were then surgically implanted under the skin of a rat for a period of 14 days while various drug treatments were administered. Following treatments, the discs were removed, fixed, sectioned and stained for analysis of vascular growth.

Disc construction

Surgical polyvinyl sponge (2 mm) thick was cut into discs using a 10 mm round metal punch. A 2 mm plug was punched out of the center of the disc and saturated with growth factor (2 μg bFGF) in an acetate copolymer (Elvax, DuPont) and placed back into the disc. The top and bottom of the discs were sealed with 10 mm diameter filter paper impregnated with Plexiglas creating impermeable top and bottom covers for the disc. The covers were glued to the disc under sterile conditions.

Surgery

Male Sprague-Dawley rats (280–300 g) were anesthetized with ketamine (100 mg/kg) and four laterally alternating surgical incisions were made on the dorsal surface. Pouches under the skin were created by gentle probing and prepared discs saturated with PBS were placed in these pouches. The incisions were closed with surgical staples. After the 14-day experimental period, the rats were sacrificed and the discs removed.

Treatments

Two days following implantation of the discs in the rat, and every other day for 2 weeks, the animals were given intramuscularly LCPT (1 mg/kg), TPT (1 mg/kg), CDDP (1 mg/kg) or TNP-470 (15 mg/kg). Empty liposomes and saline were used as control vehicles.

Disc analysis

Following removal of the disc, the impermeable covers were removed and the disc placed in 10% neutral formalin for 48–72 h. The discs were then embedded in paraffin such that 6 μm planar sections could be cut and mounted on slides. Slides were deparaffinized followed by hydration in ethanol of decreasing dilution with water (100–50%). The slides were then stained with 0.33 mg/ml toluidine blue for 30 s to permit visualization of the area of the disc penetrated by migrating cells, followed by three washes in water and then dehydrated by reversal of the alcohol washes. The slides were then rinsed with Clear Rite 3 and a coverslip mounted. The total area of the disc in each section was calculated, as was the area containing stained cells using a computerized image analysis system (Imaging Research, St. Catherine, Ontario, Canada).

Statistical analyses

The results are expressed as means ± SE ($n = 8$). Statistically significant differences between means for the DAS model were determined using the paired Student's *t*-test. Significant differences between groups were determined using a *P*-value of ≤0.001.

Results and discussion

We have previously shown that CPT inhibits DNA synthesis in normal endothelial cells, at noncytotoxic concentrations [5, 23]. In order to gain a better understanding of the growth inhibitory effect of topoisomerase I inhibitor drugs on normal endothelial cells, the effects of CPT and TPT treatment on both the level and the duration of growth and proliferation inhibition were determined. Data from the crystal violet assay (Fig. 1A) show that treatment of HUVECs with 50 n*M* CPT or 50 n*M* TPT for 24 h resulted in growth inhibition that lasted for 96 h. This inhibition was maintained even in the presence of excess (30 ng/ml) angiogenic factors (bFGF, VEGF). Furthermore, the cells in these experiments remained viable (90%) as detected by the trypan blue exclusion assay (data not shown). Incorporation of ^{3}H-thymidine into the cells over the same time and with the same treatment showed that DNA synthesis was also halted for 96 h (Fig. 1B).

These results show that the treatment of HUVECs with 50 n*M* CPT or TPT results in growth inhibitory, but not cytotoxic, effects. The inhibition of DNA synthesis occurs very rapidly. ^{3}H-thymidine uptake appeared to be inhibited by 50% in the first few minutes of treatment and gradually decreased over 24 h to complete inhibition, i.e. a biphasic response. It is possible that the first portion of the curve (Fig. 1B, inset) resulted from the inhibition of replicons that were about to initiate, whereas the shallow component of the curve corresponds to an effect on chain elongation in replicons that had already initiated. We are currently investigating this further.

Because components of angiogenesis such as endothelial cell proliferation, migration, tube formation and production of plasminogen activators and collagenase [15] in vitro do not always correlate with angiogenesis in vivo, we tested the antiangiogenic potential of CPT and TPT using an in vivo rat disc angiogenesis system (the DAS model) [28, 31]. For this type of study, the chick embryo chorioallantoic membrane (CAM) [2, 26] and the rabbit or rat cornea [19] are the most widely used systems. However, because we were able to directly evaluate the effects of CPT and TPT (administered systemically) on bFGF-induced vascular growth, we believe the DAS to be a more clinically relevant system that allows easy and accurate quantitation of angiogenesis.

In this study we compared the antiangiogenic effects of sublethal doses of CPT and TPT (1 mg/kg) to that of TNP-470 (15 mg/kg) injected intramuscularly every 2 days for 2 weeks. TNP-470 has demonstrated potent antiangiogenic activity [24, 30] and broad antitumor activity in preclinical studies [36, 41] that have warranted its testing in clinical trials [29]. Thus we used it in our study as a positive control for angiogenesis inhibition. Additionally, cisplatin which is a nonspecific cytotoxic agent was also injected intramuscularly as a negative control. Systemic administration of cisplatin at 1 mg/kg every 2 days for 2 weeks, has been shown not to produce undesirable side effects in rats [12, 27]. Thus its antiangiogenic activity was tested in this model in comparison to that of LCPT and TPT.

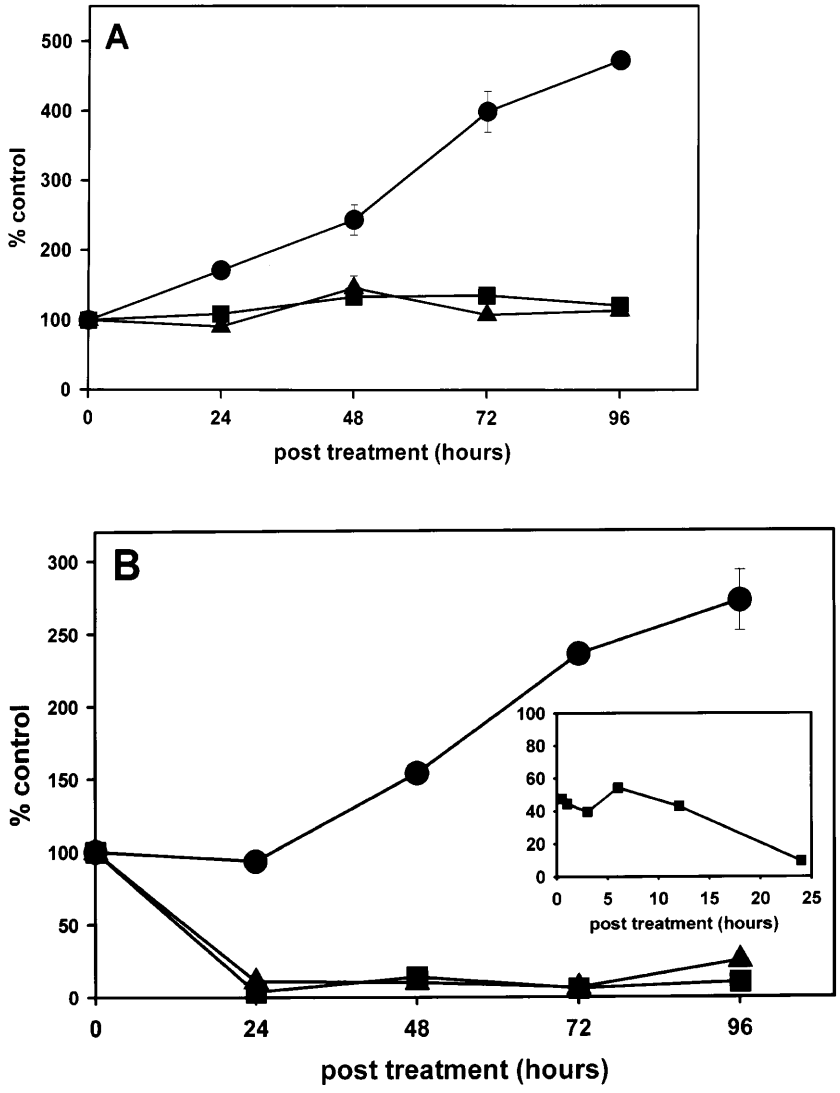

Fig. 1A, B Growth Inhibition and antiproliferative activity of CPT and TPT HUVECs were plated (2×10^4 cells/well) into a 24-well plate and allowed to attach for 24 h followed by treatment with 50 n*M* CPT (■) or TPT (▲) for 24 h. Control cells (●) were treated with an equal amount of vehicle. The cells were then washed twice with PBS and incubated in fresh medium. Plates were then assayed for crystal violet staining **(A)** and ^{3}H-thymidine uptake **(B)** at 0, 24, 48, 72, and 96 h posttreatment. ^{3}H-thymidine uptake was also measured at several time-points between 0 and 24 h during treatment (*inset*). Points are means ± SE, $n = 8$

As illustrated in Fig. 2, systemic administration of LCPT or TPT caused about a 30% inhibition of vascular growth into discs implanted in the rats ($P < 0.001$). This inhibition compared well with the inhibitory effect caused by the administration of TNP-470. In contrast, systemic administration of subtoxic doses of cisplatin did not produce any change in vascular growth into the discs. This clearly indicates that CPT and TPT inhibit not only endothelial cell proliferation in vitro, but also bFGF-induced in vivo angiogenesis. It should be noted that these results do not support the idea that CPT, TPT or TNP-470 exert their antiangiogenic effect through suppression of bFGF since the addition of excess bFGF did not reverse the inhibitory effect of CPT or TPT on in vitro endothelial cell proliferation (Fig. 1A, B).

The only known cellular targets for CPT and analogs is mammalian topoisomerase I (TopoI) [33]. These agents trap TopoI-cleavable complexes (TopoI-associated DNA single-strand breaks) by inhibiting their religation, and as a consequence generate DNA damage

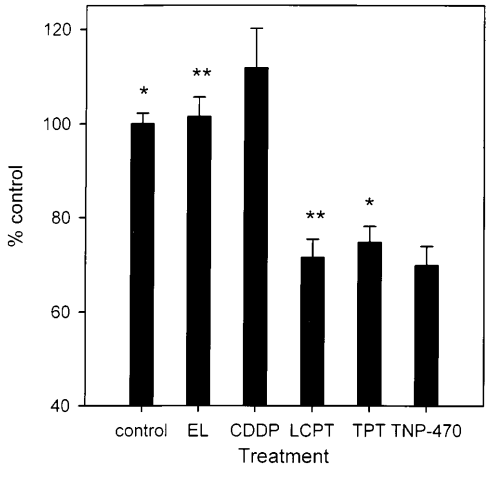

Fig. 2 Inhibition of bFGF-induced angiogenesis in the rat DAS model with the use of CPT (1 mg/kg), TPT (1 mg/kg), CDDP (1 mg/kg) and TNP-470 (15 mg/kg): Values are means ± SE of eight determinations ($P \leq 0.001$) comparing EL to LCPT and control to TPT

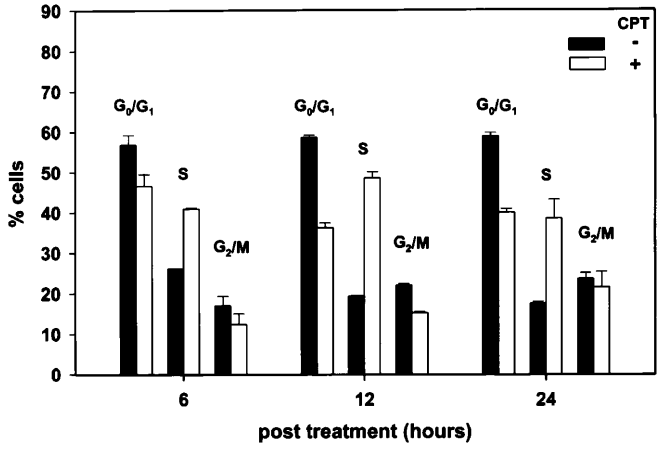

Fig. 3 Cell cycle analysis of HUVECs following incubation with CPT for 24 h. Exponential growing cultures of HUVECs were incubated with 50 n*M* CPT for 24 h and were analyzed for DNA content with a Beckton-Dickinson fluorescence-activated cell analyzer using the SFIT analysis programs provided by the manufacturer, as described in Materials and methods. The results are normalized to the control values of cells at each time-point and are the means of two or three determinations ± SE

[21]. Recent work by Coll et al. [6] has shown that multiprotein DNA replication complex (MRC)-intact TopoI activity is inhibited by 50% at 50 n*M* CPT, indicating that the MRC could be a target for inhibition of DNA replication. In this study we showed that treatment of HUVECs with 50 n*M* CPT caused about 95% inhibition of DNA synthesis that persisted for 96 h. This could be a direct effect on the MRC. This idea is further supported by the cell cycle effect of CPT treatment of HUVECs. As indicated in Fig. 3, exposure of HUVECs to 50 n*M* CPT resulted in a slowdown in the rate of cell progression through S phase leading to an increase in the S phase population (40–60% compared to 25% control) at 6, 12 and 24 h posttreatment. Similar cell cycle observations have been reported in other normal proliferating cells [1, 10].

CPT appears to have a biphasic effect on DNA synthesis (Fig. 1) with the second phase lasting up to 96 h. This effect could be due to the release of natural antiangiogenic effects that may result from inhibition of DNA replication. For example, thrombospondin-1 (TSP-1), which is secreted by numerous cell types including endothelial cells, enhances the growth of smooth muscle cells and fibroblasts, whereas it inhibits endothelial cell proliferation and angiogenesis in vitro and in vivo [3, 22]. TSP-1 has been shown to be regulated by the p53 oncoprotein, which when upregulated as a result of inhibition of DNA replication can cause the release of this naturally occurring inhibitor of angiogenesis [8, 37]. Further experiments are ongoing to unravel the precise antiangiogenic mechanism of CPT and TPT.

The results of this investigation show that CPT and TPT elicit potent, noncytotoxic, antiproliferative activity on human endothelial cells in vitro, and that this activity cannot be overcome by the addition of exogenous angiogenic growth factors. These results also show that the antiproliferative action is very rapid, with effects seen within 5 min of treatment, and the maximum effects are seen within 24 h and last for several days following removal of the drug. LCPT and TPT also showed good in vivo antiangiogenic effects in the rat DAS model and compare favorably with other antiangiogenic compounds such as TNP-470. These results taken collectively suggest that CPT and TPT may be valuable therapeutic agents for the treatment of angiogenic tumors and further investigation is warranted.

References

1. Aller P, Rius C, Mata F et al. (1992) Camptothecin induces differentiation and stimulates the expression of differentiation-related genes in U-937 promonocytic leukemia cells. Cancer Res 52: 1245
2. Auerbach R, Kubai L, Knighton D, Folkman J (1974) A simple procedure for the long term cultivation of chicken embryos. Dev Biol 41: 391
3. Bagavandoss P, Wilks JW (1990) Specific inhibition of endothelial cell proliferation by thrombospondin. Biochem Biophys Res Commun 170: 867
4. Candal FJ, Bosse DC, Vogler WR, Ades EW (1994) Inhibition of induced angiogenesis in a human microvascular endothelial cell line by ET-18-OCH_3. Cancer Chemother Pharmacol 34: 175
5. Clements MK, Wasi S, Daoud SS (1996) Camptothecin exhibits selective cytotoxicity towards human breast carcinoma as compared to normal bovine endothelial cells in vitro. Anticancer Drugs 7: 851
6. Coll JM, Hickey RJ, Wei Y, Malkas LH (1996) The human cell multiprotein DNA replication complex (MRC): the effect of camptothecin on its ability to support in vitro DNA synthesis. Cancer Chemother Pharmacol 39: 97
7. D'Amato RJ, Loughnan MS, Flynn E, Folkman J (1994) Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci USA 91: 4082
8. Dameron KM, Volpert OV, Tainsky MA, Bouck N (1994) Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. Science 265: 1582
9. Daoud SS, Fetouh MI, Giovanella BC (1995) Antitumor effect of liposome-incorporated camptothecin in human malignant xenografts. Anticancer Drugs 6: 83
10. Del Bino GJ, Skierski J, Darzyniewicz Z (1991) The concentration-dependent diversity of effects of DNA topoisomerase I and II inhibitors on the cell cycle of HL-60. Exp Cell Res 195: 485
11. Denkamp J (1993) Angiogenesis, neovascular proliferation and vascular pathophysiology as targets for cancer therapy. Br J Radiol 66: 181
12. Engineer M, Brown NS, Ho DHW, Newman RA, Bulger RE (1989) A comparison of the effects of tetraplatin and cisplatin on renal function and gentamicin pharmacology in rats. Toxicology 59: 151
13. Fan TP, Jagger R, Bicknell R (1995) Controlling the vasculature: angiogenesis, anti-angiogenesis and vascular targeting of gene therapy. Trends Pharmacol Sci 16: 57
14. Folkman J (1990) What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 82: 4
15. Folkman J, Hausdenschild C, Zetter B (1979) Long term culture of capillary endothelial cells. Proc Natl Acad Sci USA 76: 5217
16. Fotiss T, Zhang Y, Pepper MS, Adlercreutz H, Montesano R, Nawroth PP, Schweigerer L (1994) The endogenous estrogen metabolite 2-methoxyestradiol inhibits angiogenesis and suppresses tumor growth. Nature 368: 237

17. Fox BS, 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
18. Gasparini G, Harris AL (1995) Clinical importance of the determination of tumor angiogenesis in breast carcinoma: much more than a new prognostic tool. J Clin Oncol 13: 765
19. Gimbrone M, Cotran R, Folkman J (1974) Tumor growth neovascularization: an experimental model using rabbit cornea. J Natl Cancer Inst 52: 413
20. Hoffman R, Paper DH, Donaldson J, Vogl H (1996) Inhibition of angiogenesis and murine growth by laminarin sulphate. Br J Cancer 73: 1183
21. Hsiang YH, Lihou MG, Liu LF (1989) Arrest of DNA replication by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. Cancer Res 49: 5077
22. Iruela-Arispe ML, Bornstein P, Sage EH (1991) Thrombospondin exerts an antiangiogenic effect on cord formation by endothelial cells in vitro. Proc Natl Acad Sci USA 88: 5026
23. Jones CB, Clements MK, Wasi S, Daoud SS (1997) Sensitivity to camptothecin of human breast carcinoma and normal endothelial cells. Cancer Chemother Pharmacol 40: 475
24. Kim KJ, Li B, Winter J, Armanini M, Gillett N, Philips HS, Ferrara N (1993) Inhibition of vascular endothelial growth factor-induced angiogenesis-suppressed tumor growth in vivo. Nature 362: 841
25. Klauber N, Parangi S, Flynn E, Hamel E, D'Amato RJ (1997) Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyestradiol and Taxol. Cancer Res 57: 81
26. Knighton D, Ausprunk K, Tapper D, Folkman J (1977) Avascular and vascular phases of tumor growth in the chick embryo. Br J Cancer 35: 347
27. Kobayashi H, Hasuda K, Aoki K, Taniguchi S, Baba T (1990) Systemic chemotherapy in tumor-bearing rats using high-dosing cis-diamminedichloroplatinum(II) with low nephrotoxicity in combination with angiotensin II and sodium thiosulfate. Int J Cancer 45: 940
28. Kowalski J, Kwan HH, Prionas SD, Allison AC, Fajardo LF (1992) Characterization and applications of the disc angiogenesis system. Exp Mol Pathol 56: 1
29. Kudelka AP, Levy T, Verschraegen CF et al. (1997) A phase I study of TNP-470 administered to patients with advanced squamous cell cancer of the cervix. Clin Cancer Res 3: 1501
30. Kusada M, Sudo K, Fujita T, Marui S, Itoh F, Ingber D, Folkman J (1991) Potent anti-angiogenic action of AGM-1470: comparison to the fumagillin parent. Biochem Biophys Res Commun 174: 1070
31. Nelson MJ, Conley FK, Fajardo LF (1993) Application of the disc angiogenesis system to tumor-induced neovascularization. Exp Mol Pathol 58: 105
32. O'Reily MS, Boehm T, Shing Y et al. (1997) Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell 88: 1–20
33. Pommier Y (1996) Eukaryotic DNA topoisomerase I: genome gate keeper and its intruders, camptothecins. Semin Oncol 23: 1
34. Rak JW, St Croix BD, Kerbel RS (1995) Consequences of angiogenesis for tumor progression, metastasis and cancer therapy. Anticancer Drugs 6: 3
35. Schirner M, Hoffmann J, Menrad A, Schneider MR (1998) Antiangiogenic chemotherapeutic agents: characterization in comparison to their tumor growth inhibition in human renal cell carcinoma models. Clin Cancer Res 4: 1331
36. Tanaka T, Konno H, Matsuda I, Nakamura S, Baba S (1995) Prevention of hepatic metastasis of human colon cancer by angiogenesis inhibitor TNP-470. Cancer Res 55: 836
37. Volpert OV, Stellmach V, Bouck N (1995) The modulation of thrombospondin and other naturally occurring inhibitors of angiogenesis during tumor progression. Breast Cancer Res Treat 36: 119
38. Vukanovic J, Passanti A, Hirata T, Traystman RJ, Hartley AB, Isaacs JT (1993) Antiangiogenic effects of the quinoline-3-carboxamide linoamide. Cancer Res 53: 1833
39. Wellstein A, Zugmaier G, Califano JA, Kern F, Paik S, Lippman ME (1991) Tumor growth dependent on Kaposi's sarcoma-derived fibroblast growth factor inhibited by pentosan polysulfate. J Natl Cancer Inst 83: 716
40. Yamamoto T, Sudo K, Fujita T (1994) Significant inhibition of endothelial cell growth in tumor vasculature by an angiogenesis inhibitor, TNP-470 (AGM-1470). Anticancer Res 14: 1
41. Yanas T, Tamura M, Fujita K, Kodama S, Tanaka S (1993) Inhibitory effect of angiogenesis inhibitor TNP-470 on tumor growth and metastasis of human cells lines in vitro and in vivo. Cancer Res 53: 2566