The Chemopreventive Agent Oltipraz Possesses Potent Antiangiogenic Activity in Vitro, ex Vivo, and in Vivo and Inhibits Tumor Xenograft Growth

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ABSTRACT

Angiogenesis plays a pivotal role in tumor growth and represents a key target for chemopreventive intervention. On the basis of the structural features and lack of target organ specificity of the synthetic dithiolethione oltipraz, inhibition of angiogenesis was assessed as a potential mechanism for its broad-based chemopreventive activity. The effects of oltipraz on the development and maturation of a vascular network was determined in vitro using two-dimensional capillary tube formation assays with human umbilical vein endothelial cells plated on Matrigel and ex vivo using primary rat aortic ring explant cultures in three-dimensional collagen gels, respectively. The antiangiogenic and antitumor efficacy of oltipraz administration in vivo in nude mice was evaluated by determining its effects on neovascularization in s.c. Matrigel implants seeded with vascular endothelial cells and on tumor growth and angiogenesis in SVR murine angiosarcoma xenografts implanted s.c. A dose-dependent reduction in tumor growth and angiogenesis in SVR murine angiosarcoma xenografts for 10 days resulted in a significant inhibition of tumor growth as early as day 4 of dosing, with a maximum inhibition of tumor growth (81%, P < 0.001) relative to vehicle-treated mice by day 10. The observed efficacy of oltipraz in this model is comparable with that of SU 5416 and TNP-470, known antiangiogenic agents currently under clinical development. Plasma levels of oltipraz at the termination of in vivo efficacy studies were 66.4 ± 7 μM as determined by reversed phase high-performance liquid chromatography, a concentration range associated with significant antiangiogenic activity of oltipraz in vitro and ex vivo. These data suggest that the chemopreventive agent oltipraz may be effective in the treatment of advanced stage cancers and metastases, in part, because of its antiangiogenic activity in vivo.

INTRODUCTION

Angiogenesis, the development of new blood vessels from the endothelium of a preexisting vasculature, is a critical process required by solid tumors to support their localized growth and metastatic dissemination within the host. The angiogenic process entails the proliferation and migration of a normally quiescent endothelium, the controlled proteolysis of the pericellular matrix, and the synthesis of new extracellular matrix components by developing capillaries. The establishment of new intra- and intercellular contacts and the morphological differentiation of endothelial cells to capillary-like tubular networks provide support for their subsequent maturation, branching, remodeling, and selective regression to form a highly organized, functional microvascular network. The autocrine, paracrine, and amphicrine interactions of the vascular endothelium with its surrounding stromal components, as well as with the proangiogenic and angiostatic cytokines and growth factors orchestrating physiological angiogenesis, are tightly regulated both spatially and temporally (1–4). In contrast, the pathological angiogenesis necessary for active tumor growth is sustained and persistent, with the initial acquisition of the angiogenic phenotype being a common mechanism for the development of a variety of solid and hematopoietic tumor types (1–4). Tumors unable to recruit and sustain a vascular network remain dormant asymptomatic lesions in situ (4). Antiangiogenesis therapies directed against the tumor-associated endothelium and the multiple molecular and cellular processes and targets implicated in sustained pathological angiogenesis are under active evaluation for their safety and efficacy in multiple clinical trials (reviewed in Refs. 1, 4, and 5).
Chemoprevention strategies are directed at preventing the initial appearance of preneoplastic lesions or delaying or reversing their development to an invasive phenotype. In this regard, specific chemopreventive agents may directly or indirectly influence tumor-associated angiogenesis by delaying its onset (angiogenic switch) or blocking the sustained and focal neovascularization characteristic of multiple tumor types. Oltipraz [5-(2-pyrazinyl)-4-methyl-1, 2-dithiol-3-thione] is a synthetic dithiolethione with physiochemical similarities to the dithiol-thione antioxidants found among members of the Cruciferae (6). Preclinical evaluation of oltipraz has demonstrated its broad efficacy in inhibiting the development and progression of a variety of carcinogen-induced rodent tumors of multiple organ sites, including the breast, bladder, colon, stomach, liver, lymph nodes, lung, pancreas, and skin (reviewed in Ref. 7). Clinical studies on the initial use of oltipraz as an antischistosomal agent (8), and in more recent Phase I and II trials in patient populations susceptible for cancer of the colon, breast, liver, and lung (reviewed in Ref. 7), have revealed its minimal toxicity in man. The significant chemopreventive activity of oltipraz in vivo (7) and in vitro (9) has been attributed primarily to its pronounced induction of a battery of Phase II detoxification enzymes (10–13) and its activity in decreasing the formation of DNA-carcinogen adducts in vitro and in vivo (11, 14–16). To date, there are no reports of antiangiogenic activity associated with oltipraz exposure in vitro or in vivo. Preliminary microarray studies demonstrated the ability of oltipraz to modulate a number of angiogenic factors in vivo. Additional support for its potential antiangiogenic activity was suggested by the lack of target organ specificity, minimal toxicity, and specific physiochemical properties of oltipraz. On the basis of these observations, we investigated whether the antitumor efficacy profile of oltipraz in various preclinical models could be attributable, in part, to its potential antiangiogenic activity. In this study, we describe the antiangiogenic activities of oltipraz in specific in vitro, ex vivo, and in vivo models at physiological concentrations associated with its known chemopreventive properties.

MATERIALS AND METHODS

Cell Lines and Reagents. HUVECs4 were obtained from Clonetics (San Diego, CA) and cultured in EBM-2 (Clonetics) with 2% heat-inactivated fetal bovine serum (Life Technologies, Inc., Grand Island, NY), 50 μg/ml endothelial cell growth supplement, 50 μg/ml heparin, 10 mM HEPES, and 2 mM L-glutamine. Cells between passages 3 and 8 were used as described below for in vitro capillary tube assays. PAECs were obtained from the American Type Culture Collection (Rockville, MD) and grown in Ham’s F-12 medium with 10% fetal bovine serum and supplements (17) and used between passages 3 and 5 as described below. The SVR angiosarcoma cell line was obtained from the American Type Culture Collection and maintained as described (18). Cells were MAP-16 and Mycoplasma tested by a commercial laboratory (Bio Reliance Corp., Rockville, MD) and deemed suitable for in vivo studies.

Oltipraz was obtained from Rhone-Poulenc (Vitry-sur-Seine, France) and stored in powdered form in an amber vial at ambient temperature before reconstitution in DMSO (for in vitro studies) or Tw:PG (1:1 volume for volume) for p.o. administration to mice. The antiangiogenic agent TNP-470 (19) was obtained from Takeda Chemical Industries, Ltd. (Osaka, Japan) and stored dry at −20°C. Suspensions of TNP-470 in 5% ethanol and 5% arabic gum in sterile saline were prepared as described (20) before p.o. administration to mice. The 3-substituted indol-2-one VEGF-R kinase inhibitor, SU 5416 (21, 22), was synthesized and purified by Cephalon, Inc. (West Chester, PA) for use as a reference antiangiogenic agent for in vivo studies and stored in lyophilized form until reconstitution (in DMSO) immediately before use.

Animals. Female athymic nude mice (6–8 weeks old; Charles River, Wilmington, MA) were maintained five per cage in microisolator units on a standard sterilizable laboratory diet (Teklad Labchow; Harlan Teklad, Madison, WI). Animals were housed under humidity- and temperature-controlled conditions, and the light/dark cycle was set at 12-h intervals. Mice (22–24 grams) were quarantined 1 week before experimental manipulation. Male Sprague Dawley rats (250–300 grams) were obtained from Charles River and housed in a conventional vivarium facility. Male ICR (Hsd) mice (25 grams) were purchased from Taconic Farms (Germantown, NY) and housed at Fox Chase Cancer Center under standard conditions. All in vivo studies were approved by the Institutional Animal Care and Use Committees of the respective institutions.

In Vitro Capillary Tube Formation Assay with HUVECs on Matrigel. Capillary tube formation by human endothelial cells was examined in vitro by a modification of methods described previously (23, 24). Forty-eight-well Nunclon plates (Fisher Scientific, Malvern, PA) were coated with Matrigel (200 μl at 10 mg/ml; Collaborative Research, Bedford, MA) and incubated at 37°C for 30 min to promote gelling. HUVECs were cultured in EBM-2 medium (Clonetics) with 2% fetal bovine serum (Life Technologies, Inc.), and cells between passages 3 and 8 were seeded (at 3 × 10^4 cells in 200 μl of medium) in each of the Matrigel-coated wells. Oltipraz was dissolved in DMSO and added to the media in the Matrigel-coated wells at the same time as HUVECs at final concentrations of 0.4, 4, and 100 μM based on its aqueous solubility and previously published data (9, 13, 15). The final DMSO concentration in the wells was 0.02%. After an 18-h incubation at 37°C in a 5% CO2 humidified atmosphere, HUVECs were aspirated of media, fixed, and stained using a modified Wright-Giemsa staining protocol according to the manufacturer’s recommendations (Diff-Quik Stain Set; Baxter Healthcare Corp., McGraw Park, IL). Complete capillary tube networks within a designated area of a low-magnification (×10) field were counted under light microscopy, and the data were expressed as the percentage of complete capillary tube formation relative to untreated HUVEC control cultures incubated under the same conditions. All assays were done in quadruplicate in three independent experiments. Statistical analysis of inhibition of capillary tube formation

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4The abbreviations used are: HUVEC, human umbilical vein endothelial cell; PAEC, porcine aortic endothelial cell; VEGF-R, vascular endothelial growth factor receptor; FGF-R, fibroblast growth factor receptor; EBDM, endothelial cell basal medium; Tw:PG, Tween 80: propylene glycol; HPLC, high-performance liquid chromatography; IGF, insulin-like growth factor.
relative to control cultures was done by the Dunnet’s Multiple Comparison test with \( P < 0.05 \) designated as significant.

*Ex Vivo* Rat Aortic Ring Explant Assay in Collagen Gel Matrices. Rat aortic ring explant cultures were prepared by a modification of protocols described previously (24–26). Briefly, male Sprague Dawley rats (250 grams) were euthanized with CO\(_2\), and their thoracic aortas were removed aseptically and carefully to avoid damaging the vessel wall. Aortas were rinsed in serum-free EB M (Clonetics), the surrounding adventitia were removed surgically, and the cleaned vessel was cut into 1–2-mm concentric rings. Each ring was embedded in freshly prepared rat tail collagen as detailed (26). After gelation for 30 min at 37°C, collagen gel cultures were transferred to 16-mm wells (4-well NUNC dishes), each containing 0.5 ml of serum-free EB M. Oltipraz was dissolved in DMSO and mixed with serum-free EB M at final concentrations of 0.4, 4, 40, and 100 \( \mu \)M immediately before the addition or replacement of media to collagen-embedded aortic ring explant cultures. The final DMSO concentration in treated and control cultures was 0.02%. Cultures were incubated at 35.5°C in a humidified CO\(_2\) atmosphere, and the media was replaced daily over the course of the 8–10-day studies. Visual counts of microvessel outgrowths from replicate explant cultures (\( n = 4 \)) were done under bright-field microscopy following an established protocol (26). Experiments were done three times, and microvessel counts in oltipraz-treated and control cultures were analyzed by one-way ANOVA and the Student-Newman-Keuls Multiple Comparison test, with \( P < 0.05 \) deemed significant.

PAEC-VEGF-Matrigel Implant Model of Angiogenesis in Nude Mice. The Matrigel plug implantation assay used in these studies was a modification of that described by Passaniti et al. (27). Briefly, PAECs were grown to confluence in Ham’s F-12 medium supplemented with 10% fetal bovine serum. Cells were used between passages 5 and 10. Nude mice were injected bilaterally s.c. with 0.5 ml of Matrigel synthetic basement membrane (Collaborative Research) containing 1 \( \times \) 10\(^6\) PAECs/plug and recombinant murine VEGF (R&D Systems, Inc., Minneapolis, MN) at 20 ng/ml final concentration/plug. Mice bearing PAEC-VEGF-Matrigel implants were randomized into groups (10 mice/group). p.o. administration of oltipraz and the Tw:PG vehicle was done according to the dosing regimen described above in evaluating angiogenesis inhibition *in vivo* in the PAEC-VEGF-Matrigel implant model and for a total of 10 days. The fumagillin analogue, TNP-470 (19, 30), and the VEGF-R kinase inhibitor, SU 5416 (21, 22), were evaluated in this angiosarcoma model as reference antiangiogenic agents. Tumor volumes were determined with vernier calipers every 3–4 days, and both absolute tumor volumes and relative tumor volumes (normalized to individual tumor volumes at the initiation of dosing) were calculated, the latter to assess changes in the rate of tumor growth with treatment. Statistical analyses of tumor data were done using the Mann-Whitney rank sum test or, when appropriate for the data set, by one-way ANOVA and the Dunnet’s Multiple Comparison test, with \( P < 0.05 \) deemed significant.

Analysis of Oltipraz in Plasma Samples from *in Vivo* Studies. Blood was collected by aortic puncture from nude mice 30 min after the final dose of oltipraz and collected into heparinized tubes. Plasma samples were obtained by centrifugation (6 min at 8000 rpm) and stored at \(-70°C\) until analysis by reversed phase HPLC. Plasma samples were extracted and analyzed chromatographically by a modification of methods described previously (31). Briefly, 100 ml of mouse plasma sample was extracted with 300 ml of cyclopentane with gentle mixing in glass tubes for 15 min followed by centrifugation for 10 min at 3500 \( \times \) g. Samples were placed at \(-80°C\) for 10 min to facilitate removal of the upper organic phase into clean glass tubes. The organic phase was dried under a nitrogen stream at 42°C for 20 min. The residue was reconstituted with 30 ml of 50% methanol for subsequent HPLC analyses. Anethole tri- thione was used as an internal standard for these analyses as described (31), and a purified standard of oltipraz was added to samples of naïve mouse plasma as additional controls for ex-
traction efficiency. A 44% extraction efficiency for oltipraz was obtained from spiked mouse plasma samples.

The HPLC system consisted of a Hewlett-Packard HP1046A liquid chromatograph equipped with an autoinjector and diode-array UV detector. The detector wavelength was set initially at 290 nm and switched to 302 nm at 4 min and to 248 nm at 8 min for each chromatographic evaluation to optimize the analyses. Chromatographic separation was achieved on a C8 reversed phase column (Adsorosphere, 5 m; 150 × 4.6-mm inner diameter; Alltech Associates, Deerfield, IL). An isocratic separation was achieved using a mobile phase of 58% 0.005 M dimethyloctylamine, 0.5 mM sodium acetate (pH 5.5), and 42% acetonitrile at a flow rate of 1.5 ml/min. The retention time for oltipraz was 5 min, and calibration curves were linear over the concentration range described previously (31).

cDNA Expression Arrays. ICR (H) mice were randomized to receive either oltipraz (1 gram/kg) or vehicle (1% carboxymethylcellulose: 25% glycerol) by intragastric gavage (four mice per group). The oltipraz dose was selected to achieve maximal induction of gene expression and is based on a previous in vivo characterization of oltipraz (12). Animals were sacrificed at 6 and 24 h post-treatment. Livers were excised, snap frozen in liquid nitrogen, and stored at −80°C until the time of analysis. Total hepatic RNA was isolated according to established protocols (32). RNA was treated with DNase I (Roche Diagnostic Corp., Indianapolis, IN) and subjected to repeated phenol:chloroform:isoamyl alcohol extractions, ethanol precipitations, and washings. The quality of the resulting RNA was assessed by absorbance at 260 and 280 nm and visualization after electrophoretic separation on a 10% formaldehyde gel.

Drug-induced changes in gene expression were characterized using the Atlas cDNA expression array containing cDNAs representing 588 mouse genes (Clontech Laboratories, Inc., Palo Alto, CA). Preparation of the 32P labeled complex cDNA probe was performed according to the manufacturer’s instructions, except that a modified nonradioactive dCTP was included in the reaction mixture. The modified dCTP is highly susceptible to cleavage when the Ambion Strip-E2 kit (Ambion, Austin, TX) is used, thus ensuring complete stripping of the blot between experiments, as confirmed by autoradiography. Atlas membranes were prehybridized, hybridized, washed, and developed using conditions recommended by the manufacturer.

X-ray films, containing “control” and “treated” blots on the same film, were scanned using a Hewlett-Packard imagescanner at a 1200 dpi resolution, in 256K grayscale. Images were imported into the Scion Image Program (Scion, Frederick, MD). The mean pixel density of the background area was subtracted from the mean pixel density of duplicate images, representing each gene, and the area measured multiplied the resulting total.

RESULTS AND DISCUSSION

Antiangiogenic Activity of Oltipraz on Microvessel Growth In Rat Aortic Ring Explant Cultures Ex Vivo and Capillary Tube Formation by HUVECs in Vitro. The dose-related effects of oltipraz on microvessel growth in rat aortic ring explant cultures embedded in collagen gels are shown in Fig. 1. Angiogenesis in this model is a self-limiting physiological process mediated in response to the injury of the aortic dissection process and occurring in the absence of exogenous addition of serum, VEGF, or other angiogenic cytokines to the culture medium (33, 34). This assay enables a quantitative assessment of microvessel growth, branching and remodeling, and vessel regression in an explant culture system, in which endothelial cell-adventitial cell interactions critical for angiogenesis in vivo can be effectively modeled in an ex vivo setting.

As shown in Fig. 1, administration of 40 and 100 μM oltipraz inhibited microvessel growth by 50 (% < 0.05) and 95% (% < 0.01), respectively, relative to vehicle-treated control cultures during the peak (angiogenic) phase of microvessel growth (day 8) in this explant culture assay. Lower concentrations of oltipraz (0.1, 0.4, and 4 μM) were not inhibitory in this model. There were no apparent cytotoxic effects of oltipraz over the concentration range evaluated on the endothelial cells, fibroblasts, pericytes, and smooth muscle cells present in these aortic ring explant cultures based on trypan blue exclusion (data not shown). The inhibition of microvessel growth by oltipraz was observed over a physiologically relevant range of oltipraz concentrations capable of inducing Phase II detoxification enzymes and inhibiting DNA-carcinogen adduct formation in vitro (13, 15, 16) but not lower concentrations of oltipraz shown recently to have chemopreventive activity in vitro (35).

To evaluate the potential antiangiogenic activity of oltipraz
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HUVEC cultures, vehicle-treated control cultures (left panel the control. Duplicate experiments were run with four replicates. Vari-

ation in tube formation was ±5–9% across replicates. * = P < 0.05 by Dunnet’s Multiple Comparison test. B, light microscopy photographs of HUVEC cultures, vehicle-treated control cultures (left panel), and cultures treated with 100 μM oltipraz (right panel). Magnification: ×100.

in a bioassay using human endothelial cells, its ability to inhibit capillary tube formation by HUVECs grown on a Matrigel synthetic extracellular matrix was examined (Fig. 2). Under these conditions, HUVECs are capable of morphological differentiation into an extensive network of capillary-like structures composed of highly organized three-dimensional cords (36). This assay models specific critical steps in the earlier phases of an in vivo angiogenic response, including endothelial cell adhesion, basement membrane proteolysis, and the migration and differentiation of endothelial cells into a network of capillary tube-like structures: events preceding the formation of new functional microvessels in vivo (23, 24). As shown in Fig. 2, oltipraz inhibited complete capillary tube formation by HUVECs by 23, 62 (P < 0.05), and 52% (P < 0.05) relative to vehicle-treated control cultures at concentrations of 4, 40, and 100 μM, respectively, in the absence of cytotoxicity to human endothelial cells in culture based on trypan blue exclusion. Concentrations of oltipraz <4 μM did not have any significant antiangiogenic activity in this assay (data not shown). The slight reduction in inhibitory activity observed at 100 μM oltipraz in this assay may have been attributable to its limited solubility at this higher concentration under these assay conditions using Matrigel as a matrix. These data substantiate the observations made in rat aortic ring explant cultures for pronounced dose-related inhibition of angiogenesis by oltipraz in both rodent and human bioassays in the absence of cytotoxicity.

Fig. 3 Effects of p.o. oltipraz administration on angiogenesis in vivo in the PAEC-VEGF-Matrigel implant model. Female athymic nude mice were injected bilaterally s.c. with Matrigel containing 1 × 10⁶ PAEC and 20 ng/ml recombinant murine VEGF. Implanted mice were administered oltipraz by p.o. gavage at 250 mg/kg/dose qd in a Tw:PG vehicle (100 ml/dose). Six days postdosing, implants were excised and evaluated for the extent of neovascularization colorimetrically (540 nm) on the basis of hemoglobin content (Drabkin method) as detailed in “Materials and Methods.” Results from two independent experiments (n = 10 mice/group in each) are expressed as mean grams/dl of hemoglobin ± SE. *P < 0.05.

In Vivo Antiangiogenic Activity of p.o.-administered Oltipraz in the PAEC-Matrigel Plug Assay in Nude Mice. One approach to the study of angiogenesis in vivo has been the evaluation of neovascularization into biocompatible polymer matrices containing known angiogenic factor(s) or cell types implanted s.c. in vivo. This approach has provided a rapid and quantitative means to evaluate the dose-related induction or inhibition of angiogenesis by a variety of stimuli relative to untreated control matrix implants (27, 29, 37). The in vivo inhibition of angiogenesis by p.o.-administered oltipraz was evaluated in a modified polymer implant assay using PAEC and VEGF in Matrigel-injected s.c. into athymic nude mice. The determination of the extent of angiogenesis within the implanted matrix was based on the quantitation of the hemoglobin content of the vascularized implants on removal from the host. Oltipraz was administered at 250 mg/kg/dose p.o. once a day in a Tw:PG vehicle based on its pharmacokinetic profile in mice to achieve sustained plasma concentrations in a physiologically relevant (μM) range over a 6- to 24-h period (28). As shown in Fig. 3, p.o. administration of oltipraz for 6 days resulted in a 42% reduction in neovascularization (P < 0.05) relative to Tw:PG vehicle-treated control mice over the same time course. These results were observed in the absence of apparent toxicity or morbidity as assessed by body weight loss in nude mice (data not shown). By comparison, the VEGF-R kinase inhibitor, SU 5416, dosed at 12 mg/kg/dose i.p. once a day in DMSO (22) resulted in a ~60% reduction in neovascularization in this assay over the same time course. These observations are in accord with previous reports of the tolerability of oltipraz administration in rodents (7) and demonstrate the significant p.o. antiangiogenic efficacy of oltipraz in an established in vivo bioassay of physiological angiogenesis (27, 29).

Antitumor Efficacy of p.o. Oltipraz Administration on Established Murine SVR Angiosarcoma Xenografts in Athy-

mic Nude Mice. The SVR angiosarcoma model is a highly aggressive endothelial cell tumor with a high take rate and short latency period that is generally refractory to standard treatment
regimens and results in the death of the host animal from local invasiveness and hemorrhage (18, 30). Because of the nature of this tumor model, inhibition of its growth is likely to reflect both antiangiogenic activities on the host endothelium and antitumor activity on the angiosarcoma cells directly (30). p.o. administration of oltipraz at the same dose (250 mg/kg/dose p.o. qd), shown to be antiangiogenic in the Matrigel plug implant model (Fig. 3), was initiated in athymic mice bearing established s.c. SVR angiosarcoma xenografts (95 ± 5 mm³ 6 days postimplantation) and continued for a total of 10 days. As shown in Fig. 4, oltipraz administration resulted in a significant inhibition of tumor growth as early as day 4 of dosing (P < 0.005) with a maximum inhibition of tumor growth (81%, P < 0.001) relative to Tw:PG vehicle-treated mice by day 10 of the dosing regimen. These effects were observed in the absence of apparent morbidity or mortality to the oltipraz-treated mice, as there were no adverse events or significant body weight loss in these animals. By comparison, administration of SU 5416 (25 mg/kg/dose i.p. qd; Ref. 22) and TNP-470 (30 mg/kg/dose s.c. Q2D × 3; Refs. 20 and 30) in the same model over the same time course resulted in a 75 and 80% maximum reduction in SVR tumor volume, respectively, relative to vehicle-treated control mice by day 10 (Fig. 4). These results are in agreement with published accounts in this model with TNP-470 administration (30). These findings indicate that p.o. administration of oltipraz has an antitumor efficacy in the highly aggressive and angiogenic SVR angiosarcoma model comparable with that of two established antiangiogenic agents currently under clinical evaluation. Plasma concentrations of oltipraz obtained 30 min after the final dose to SVR tumor-bearing nude mice were 66.4 ± 7 μM as determined by reversed phase HPLC analysis. These plasma levels associated with significant antitumor and antiangiogenic activity in vivo are in accord with the concentration range of free oltipraz demonstrating significant antiangiogenic activity in vitro and ex vivo in the capillary tube formation and rat aortic ring explant models, respectively, in the absence of apparent cytotoxicity (Figs. 1 and 2).

The mechanism(s) by which oltipraz exerts the antiangiogenic efficacy observed in the in vitro, ex vivo, and in vivo models described here is not known. ELISA-based kinase assays examining the inhibitory profile of oltipraz against recombinant human FGF-R1, platelet-derived growth factor receptor, VEGF-R1, VEGF-R2, and VEGF-R3 receptor tyrosine kinase domains revealed the absence of significant inhibitory activity of oltipraz against these proangiogenic receptor tyrosine kinases (data not shown).

Comparison of hepatic gene expression in oltipraz- and vehicle-treated animals by cDNA array revealed the ability of oltipraz to inhibit the expression of angiogenesis-related genes (Fig. 5). Hepatic expression of the placental RNase inhibitor angiogenin and IGF-IA was reduced to 35 and 78% of vehicle-treated control, respectively, 6 h after oltipraz exposure. The biological consequences of these altered expression patterns after oltipraz exposure remains to be determined. By 24 h post-treatment, the level of transcripts for both genes approached the limits of detection in oltipraz-treated mice. A 40% decrease in VEGF-R2 expression was also observed in oltipraz-treated mice at 6 h post-treatment. However, this result should be interpreted with caution because of the weak nature of the associated signal. Expression of 11 other angiogenesis-related genes was evaluated but found to be undetectable in both controls and oltipraz-treated animals at the time points examined (Fig. 5). Gene expression patterns are likely to vary among different target tissues and specific cell types on exposure to oltipraz; consequently, we cannot conclude that these differences in hepatic gene expression in vivo may reflect unequivocally the patterns of gene expression of the inherently heterogeneous vascular endothelium in vivo (38). This caveat notwithstanding, these gene array data nonetheless provide a ra-
The effect of oltipraz on modulating the expression of proangiogenic growth factors (other than IGF-IA detected by cDNA expression array analyses), including VEGF, acidic and basic FGF, and platelet-derived growth factor receptor, or additional proangiogenic regulatory components remains to be established. Additional studies will be directed at understanding the mechanism(s) for the antiangiogenic activity of oltipraz and related dithiolthiones and elucidating the role this activity may play in the chemopreventive efficacy of this compound in multiple preclinical models of human cancer.

In summary, these data demonstrate that oltipraz has potent antiangiogenic activity at physiological concentrations that were shown previously to have minimal toxicity in humans. This antiangiogenic and antitumor activity in preclinical models was associated with plasma levels of oltipraz corresponding to concentrations demonstrating significant antiangiogenic activity in vitro and ex vivo. In view of its previously established pharmacological properties as a prototypic chemopreventive agent, oltipraz may have yet additional therapeutic and chemopreventive clinical applications.

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