**In Vivo Antitumor Activity of Bis(4,7-dimethyl-1,10-phenanthroline) Sulfatooxovanadium(IV) (METVAN [VO(SO_4)(Me_2-Phen)_2])**

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**ABSTRACT**

The compound bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium(IV) (METVAN [VO(SO_4)(Me_2-Phen)_2]), exhibits potent cytotoxicity against human cancer cells at low micromolar concentrations. At concentrations \( \geq 1 \mu M \), METVAN treatment was associated with a nearly complete loss of the adhesive, migratory, and invasive properties of the treated tumor cell populations. METVAN did not cause acute or subacute toxicity in mice at dose levels ranging from 12.5 mg/kg to 100 mg/kg. Therapeutic plasma concentrations \( \geq 5 \mu M \) were rapidly achieved and maintained in mice for at least 24 h after i.p. bolus injection of a single 10 mg/kg nontoxic dose of METVAN. At this dose level, the maximum plasma METVAN concentration was 37.0 \( \mu M \), which was achieved with a \( t_{1/2} \) of 21.4 min. Plasma samples (diluted 1:16) from METVAN-treated mice killed 85% of human breast cancer cells in vitro. METVAN was slowly eliminated with an apparent plasma \( t_{1/2} \) of 17.5 h and systemic clearance of 42.1 ml/h/kg. In accordance with its potent in vitro activity and favorable in vivo pharmacokinetics, METVAN exhibited significant antitumor activity and delayed tumor progression in CB.17 severe combined immunodeficient (SCID) mouse xenograft models of human glioblastoma and breast cancer. In these experiments, METVAN was administered in daily injections of a single nontoxic 10 mg/kg i.p. dose on 5 consecutive days per week for 4 consecutive weeks beginning the day after the s.c. inoculation of U87 glioblastoma or MDA-MB-231 breast cancer cells. At 40 days after the inoculation of tumor cells, the U87 tumor xenografts in the vehicle-treated control SCID mice were much larger than those of the mice treated with METVAN (4560 ± 654 mm\(^3\) versus 1688 ± 571 mm\(^3\); \( P = 0.003 \)). Similarly, the MDA-MB-231 tumors in SCID mice treated with METVAN were much smaller 40 days after tumor cell inoculation than those of the vehicle-treated control SCID mice (174 ± 29 mm\(^3\) versus 487 ± 82 mm\(^3\); \( P = 0.002 \)). The favorable in vivo pharmacodynamic features and antitumor activity of METVAN warrants further development of this novel oxovanadium compound as a potential new anticancer agent.

**INTRODUCTION**

The use of vanadium compounds and vanadium salts for clinical applications received renewed interest in the late 1970s and early 1980s because of the discovery that vanadate(V) solutions produced insulin-like effects in rat diaphragms and isolated adipocytes in vitro (1–3). Subsequently, the administration of vanadate solutions to diabetic rats was shown to lower blood glucose levels (4). Despite a long history of vanadium compounds being used in the treatment of human diseases, however, only a small number of organometallic compounds containing vanadium have been tested for antitumor activity (5–11); a few of these were shown to possess anticancer activity in vitro as well as in vivo (6, 8, 12–14).

Vanadium can be found in both anionic and cationic forms with oxidation states ranging from −1 to +5 (15–18). Vanadium complexes with oxidation states +4 (IV) and +5 (V) have been shown to modulate cellular redox potential, regulate enzymatic phosphorylation, and exert various other effects in multiple biological systems (19–25). In addition to the ability of vanadium to assume various oxidation states, its coordination chemistry also plays a key role in its interactions with various biomolecules. As part of a systematic effort aimed at developing compounds with anticancer activity, we recently identified METVAN, (bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium(IV) [VO(SO_4)(Me_2-Phen)_2]) as an active apoptosis-inducing agent with potent in vitro antitumor activity (26–28). At nanomolar to low micromolar concentrations, METVAN induced apoptosis in leukemia cell lines, multiple myeloma cell lines, and solid tumor cell lines derived from breast cancer, glioblastoma, and testicular cancer patients (26–28). Apoptosis induced by treatment with METVAN is mediated by the generation of reactive oxygen species, depletion of glutathione, and depolarization of mitochondrial membranes (29). Furthermore, METVAN inhibited the constitutive expression of MMP-2,9 protein and its gelatinolytic activity in HL-60 acute myeloid leukemia cells and MMP-2 as well as of MMP-9 gelatinolytic activities in leukemia cells from acute lymphoblastic leukemia.

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2 The abbreviations used are: MMP, matrix metalloproteinase; SCID, severe combined immunodeficient; IR, infrared; LC, liquid chromatography; MS, mass spectrometry; ES, electron spin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; ECM, extracellular matrix; AUC, area under the concentration-time curve; TdT, terminal deoxynucleotidyltransferase; TUNEL, TdT-mediated dUTP nick-end labeling.
acute myeloid leukemia, and chronic myeloid leukemia patients (30). We now report that concentrations of METVAN, highly cytotoxic to human glioblastoma and breast cancer cells in vitro, can be achieved in vivo after i.p. administration of a single nontoxic dose. METVAN showed favorable pharmacokinetics in mice and exhibited significant in vivo antitumor activity in SCID mouse xenograft models of human glioblastoma and breast cancer.

MATERIALS AND METHODS

Preparation and Characterization of METVAN. The coordination compound METVAN, bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium(IV) [VO(SO$_4$)(Me$_2$-Phen)$_2$], was synthesized as described previously in detail (26, 27). METVAN was characterized by IR, LC-MS, and elemental analysis. The results were as follows: (a) IR (KBr pellet), $\nu$ (cm$^{-1}$) 3421, 3066, 2924, 1624, 1608, 1578, 1524, 1423, 1383, 1297, 1237, 1157, 1030, 972, 930, 868, 729, 696, 663, 650, 590, 557, 545, 529, 485, 464; (b) LC-MS (ES), LC conditions: eluant, 30:70 v/v mixture of acetonitrile and an aqueous solution of 0.1% acetic acid; flow rate, 0.5 ml/min; column, 250 × 4 mm LiChrospher 100 RP-18 (5 μm); injection volume, 10 μl; detection, 300 nm; and LC-MS (ES), MS conditions (positive ion mode): fragmentor voltage, 50 V; drying gas flow rate, 10 liter/min; drying gas temperature, 350°C; nebulizer pressure, 25 p.s.i.; retention time, 7.84 min; ES-MS, calculated for [M + H]$^+$ 580.1 [M = [VO(SO$_4$)(Me$_2$-Phen)$_2$]]; $C_{28}$H$_{24}$N$_4$O$_5$SV; found, 580.1; and (c) elemental analysis: calculated for [VO(SO$_4$)(Me$_2$-Phen)$_2$]·2H$_2$O (C$_{35}$H$_{32}$N$_4$O$_5$S·2H$_2$O), C, 54.63; H, 4.59; and N, 9.10; found: C, 54.79; H, 4.50; N, 9.26.

Cell Lines. Human brain tumor cell lines U87 MG, U118 MG, U138, U373 MG, and T98G, and breast cancer cell lines BT-20, MDA-MB-231, MDA-MB-361, and MCF-7, were obtained from American Type Culture Collection (Rockville, MD) and were maintained as continuous cell lines in DMEM (U87, U118, U138, U373, T98, BT-20) or in Leibovitz’s L-15 medium (MDA-MB-231 and MDA-MB-361). All of the media were supplemented with 10% FCS, 4 mm glutamine, 100 units/ml penicillin G, and 100 mg/ml streptomycin sulfate. All of the tissue culture reagents were obtained from Life Technologies, Inc. (Gaithersburg, MD).

Cytotoxicity MTT Assays. The cytotoxicity of METVAN against human cancer cell lines was analyzed using the MTT assay (Boehringer Mannheim Corp., Indianapolis, IN) as described previously (27). Briefly, exponentially growing tumor cells were seeded into a 96-well plate at a density of 2.5 × 10$^4$ cells/well. METVAN was dissolved in 0.1% DMSO in PBS and then added to each well to yield final concentrations ranging from 0.1 to 250 μM. After incubation at 37°C for 48 or 16 h, 10 μl of MTT (0.5 mg/ml final concentration) were added to each well. The plates were then incubated at 37°C for an additional 4 h to allow MTT to form formazan crystals by reacting with metabolically active cells. The formazan crystals were solubilized overnight at 37°C in a solution containing 10% SDS in 0.1 M HCl. The absorbance at 540 nm (a reference wavelength of 690 nm was used) of the solution in each well was measured using a microplate reader (Labsystems). The percentage survival and the IC$_{50}$ values were calculated using Graphpad Prism v2.0 (Graphpad Software, Inc., San Diego, CA).

Adhesion Assays. In vitro adhesion assays were used to evaluate the effects of METVAN on the adhesive properties of U87 and MDA-MB-231 cells as described previously (31). The plates for the adhesion assays were precoated with the ECM proteins laminin, fibronectin, vitronectin, and type IV collagen (each at a final concentration of 1 μg/ml in PBS) overnight at 4°C and dried. Exponentially-growing cells were incubated with METVAN at concentrations ranging from 0.5 μM to 25 μM for 16 h in a humidified 5% CO$_2$ atmosphere. The cells were then detached from the flasks with 0.05% trypsin (Life Technologies, Inc.), resuspended in medium, incubated at 37°C for 2 h to allow them to recover from the trypsinization stress, and examined for their ability to adhere to the plates precoated with ECM proteins. Cells were centrifuged, washed twice with serum-free medium, counted, and resuspended in serum-free medium at a final

Fig. 1 Cytotoxic activity of METVAN against human glioblastoma and breast cancer cells. Human glioblastoma (A, U87, U118, U138, U373, and T98) and breast cancer (B, BT-20, MDA-MB-231, MDA-MB-361, and MCF-7) cells were incubated with increasing concentrations (0.1–250 μM) of METVAN for 48 h (A and B) or 16 h (C) in 96-well plates. Cell survival was determined by MTT assays. Data points, the mean (± SE) values from three independent experiments. The mean (± SE) IC$_{50}$ values for 48-h incubation were 2.1 ± 0.6 μM (U87), 0.82 ± 0.1 μM (U118), 2.7 ± 0.4 μM (U138), 2.0 ± 0.4 μM (U373), 3.9 ± 0.5 μM (T98), 1.6 ± 0.7 μM (BT-20), 0.5 ± 0.1 μM (MDA-MB-231), 1.9 ± 0.5 μM (MDA-MB-361), and 2.3 ± 0.4 μM (MCF-7). The mean (± SE) IC$_{50}$ values for 16 h incubation were 16.3 ± 1.8 μM (U87) and 11.1 ± 1.8 μM (MDA-MB-231).
concentration of $2.5 \times 10^5$ cells/ml. The cell suspension was added to each well in aliquots of 100 µl, and the cells were allowed to adhere for 1 h at 37°C in a humidified 5% CO$_2$ atmosphere. The nonadherent cells were removed by gently washing the cells with PBS. The adherent fraction was quantitated using MTT assays as described previously (27, 32).

**In Vitro Invasion Assays.** The *in vitro* invasiveness of cancer cells treated with METVAN was assayed using Matrigel-coated Costar 24-well transwell cell culture chambers (Boyden chambers) with 8.0-µm-pore polycarbonate filter inserts as described previously (31, 33, 34). Exponentially-growing U87 and MDA-MB-231 cells were incubated with METVAN at various concentrations ranging from 0.5 µM to 25 µM overnight. The cells were trypsinized, washed twice with serum-free medium containing BSA, counted and resuspended in a serum-free medium at $1 \times 10^5$ cells/ml. The cell suspension (0.5 ml aliquots) was added to the Matrigel-coated and rehydrated filter inserts. The inserts were then placed in 24-well plates containing 750 µl of NIH fibroblast-conditioned medium (31, 34) as a chemoattractant and were incubated at 37°C for 48 h. The filter inserts were then removed, the medium was decanted, and the cells on the top of the filter that did not migrate were scraped off with a cotton-tipped applicator. The invasive cells that migrated to the lower side of the filter were fixed, stained with Hema-3 solution, and counted under a light microscope. Five to 10 random fields per filter were counted to determine the invasive fraction. The invasive fractions of cells treated with METVAN were compared with those of DMSO-treated control cells and the percentage inhibition of invasiveness was determined.

**Migration Assay.** Migration of brain tumor cells was monitored using U373 glioblastoma cell spheroids, as described previously (31). Glioblastoma cell spheroids were cultured in 100-mm² tissue culture plates precoated with 0.75% agar prepared in MEM supplemented with 10% fetal bovine serum. The cells $(5 \times 10^6$ cells) were suspended in the medium, seeded onto agar-coated plates, and cultured for 5–7 days at 37°C. Spheroids with a diameter of 200–400 µm were selected for use in additional experiments. For the migration experiments, the selected spheroids were incubated for 2 h at 37°C in serum-free medium containing METVAN in concentrations ranging from 1 µM to 25 µM. The METVAN-treated and vehicle-control (DMSO, 0.1%) spheroids were transferred onto fibronectin-coated coverslips (Becton Dickinson, Bedford, MA) and placed in 6-well plates containing the same concentrations of METVAN or DMSO in serum-free medium. Spheroids were then kept in a humidified 5% CO$_2$ incubator at 37°C for 48 h. A total of four to six spheroids were used for each concentration. After the 48-h incubation period, the spheroids were fixed and stained with Hema-3 solutions and mounted onto glass slides. The distance of tumor cell migration from the spheroid was

![Image](Fig. 2) METVAN induces apoptosis in glioblastoma and breast cancer cells. U87 glioblastoma (A, A’) and MDA-MB-231 breast cancer (B, B’) cells were incubated with 10 µM of METVAN for 24 h, fixed, permeabilized and visualized for DNA degradation in a TUNEL assay using dUTP-labeling. Red fluorescence, nuclei stained with propidium iodide. Green or yellow (i.e., superimposed red and green) fluorescence, apoptotic nuclei containing fragmented DNA. When compared with controls, treated with 0.1% DMSO (A, B), several of the cells incubated with METVAN (A’, B’) exhibited apoptotic nuclei.

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measured using an ocular micrometer and a transmitted light microscope.

**Mice.** The animal protocols used in this study were approved by the Parker Hughes Institute Institutional Animal Care and Use Committee (IACUC), and all of the animal care procedures conformed to the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, Washington DC 1996). Female CB.17 SCID and CD-1 mice were obtained from Taconic (Germantown, NY) and housed in a specific-pathogen-free room located in a secure indoor facility with controlled temperature, humidity, and noise levels. Mice were housed in microisolater cages and fed with autoclaved rodent chow. Water was also autoclaved and supplemented with trimethoprim/sulfomethoxazol 3 days a week.

**Toxicity Studies in Mice.** The toxicity profile of METVAN in CD-1 mice was examined, as reported previously for other new agents (35–38). Female CD-1 mice (7 weeks old; average weight, 24.5 g) were used and monitored daily for lethargy, cleanliness, and morbidity. At the time of death, necropsies were performed, and the toxic effects of METVAN administration were assessed. For histopathological studies, tissues were fixed in 10% neutral buffered formalin, dehydrated, and embedded in paraffin by routine methods. Glass slides with affixed 6-μm tissue sections were prepared and stained with H&E. Female CD-1 mice were given an i.p. bolus injection of METVAN in 0.2 ml of PBS supplemented with 10% DMSO, or 0.2 ml of PBS supplemented with 10% DMSO alone (control mice). No sedation or anesthesia was used throughout the treatment period. Mice were monitored daily for mortality for determination of the day-30 LD50 values. Blood samples were collected prior to and after METVAN administration for a complete blood cell count (CBC) with differential and platelets, serum samples for total protein, albumin, bilirubin/ALT, alkaline phosphatase, creatinine, CPK, and electrolytes. Mice surviving until the end of the 30-days monitoring were killed, and the tissues were immediately collected from randomly selected mice and preserved in 10% neutral buffered formalin. Standard tissues collected for histological evaluation included: bone, bone marrow, brain, cecum, heart, kidney, large intestine, liver, lung, lymph node, ovary, pancreas, skeletal muscle, skin, small intestine, spleen, stomach, thymus, thyroid gland, urinary bladder, and uterus (as available).

**SCID Mouse Xenograft Models of Human Glioblastoma and Breast Cancer.** The right and left hind legs of the SCID mice were inoculated s.c. with 1 × 106 U87 human glioblastoma or 1 × 106 MDA-MB-231 breast cancer cells in 0.1 ml of PBS. Mice were treated with i.p.-administered injections of METVAN (10 mg/kg in 5% DMSO in PBS; n = 10) or vehicle alone (5% DMSO in PBS; n = 10). Injections were given once daily, 5 days per week, for 4 consecutive weeks beginning the day after inoculation of the tumors. Mice were monitored daily for health status as well as tumor growth and were killed if they became moribund or developed tumors that impeded their ability to attain food or water, or at the end of the six-week observation period. Tumors were measured using Vernier calipers three times per week. Tumor volumes were calculated according to the following formula, as described previously (32, 39): tumor volume = (width)²(length)/2.

**Pharmacokinetic Studies in Mice.** Female CD-1 mice (6–8 weeks old) from Charles River (Wilmington, MA) were housed in a controlled environment (12-h light/12-h dark photoperiod, 22 ± 1°C, 60 ± 10% relative humidity). The mice were allowed free access to autoclaved pellet food and tap water throughout the study. The animal studies are approved by the Parker Hughes Institute Animal Care and Use Committee, and all animal care procedures conformed to the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, Washington DC 1996). A solution (100 μl) of METVAN (dissolved in 5% DMSO in PBS) was administered i.p. to mice at a dose of 10 mg/kg. This volume of DMSO is well tolerated by mice when administrated by rapid i.v. or extravascular injection (40, 41). Blood samples (~500 μl) were obtained from the ocular venous plexus by retro-orbital venipuncture at 0, 2, 5, 10, 15, 30, 45 min, and 1, 2, 4, 6, and 24 h after the i.p. injection. All of the collected blood samples were heparinized and centrifuged at 7000 × g for 5 min to separate the plasma fraction from the whole blood. The plasma samples were then processed immediately, using the procedure described below for determining vanadium concentrations.

**Determination of Plasma METVAN Levels by Induc tally Coupled Plasma Atomic Emission Spectroscopy.** All of the glassware was kept in 10% nitric acid for at least 48 h and was subsequently washed with distilled and Millipore water.
before use. Standard solutions containing 0–200 μg/liter of vanadium were prepared by diluting 100 μl of plasma samples containing various concentrations of METVAN with 3.7% HCl to 5 ml. The plasma samples from mice (100 μl) were diluted with 3.7% HCl to 5 ml and were analyzed by inductively coupled plasma atomic emission (ICP) spectroscopy carried out in the Research Analytical Laboratory at University of Minnesota using a Perkin-Elmer Optima 3000DV spectrometer. The analytical wavelength was 292.396 nm with plasma gas flow of 15 liters/min, auxiliary gas flow of 0.5 liter/min and nebulizer gas flow of 0.75 liter/min. The output power was 1350 W. The reporting limit for vanadium was 0.001 μg/ml. (Reporting limit is based on the concept of the lowest quantitatively determinable concentration (LQDC). Precision at the LQDC is approximately ±10% and analytical results are quantitative.)

Pharmacokinetic Analysis. Pharmacokinetic modeling and parameter calculations were carried out using the WinNonlin Professional Version 3.0 (Pharsight, Inc., Mountain, CA) pharmacokinetics software (35, 36, 42, 43). An appropriate model was chosen on the basis of the lowest sum of weighted squared residuals, the lowest Schwartz criterion (SC), the lowest Akaike’s information criterion (AIC) value, the lowest SEs of the fitted parameters, and the dispersion of the residuals. The elimination t_{1/2} life was estimated by linear regression analysis of the terminal phase of the plasma concentration-time profile. The AUC was calculated according to the linear trapezoidal rule between the first sampling time (0 h) and the last sampling time plus C/k, where C is the concentration of the last sampling and k is the elimination rate constant. The systemic clearance (CL) was determined by dividing the dose by the AUC.

In Situ Detection of Apoptosis. Apoptosis was detected using an in situ cell death detection kit (Boehringer Mannheim

Fig. 4 Effects of METVAN on glioblastoma cell migration from spheroids. U373 glioblastoma spheroids with diameters of 200–356 μm were treated for 2 h with 1 μM (B), 2 μM (C) or 5 μM (D) METVAN in 0.1% DMSO or with 0.1% DMSO alone (A). The control cells migrated 745 ± 29 μm from the spheroids, whereas treatment of the spheroids with 1 μM (B), 2 μM (C) and 5 μM (D) METVAN resulted in migration distances of only 311.1 ± 54.4 μm; 214.9 ± 25.7 μm and 8.4 μm (corresponding to 58.3 ± 7.3%, 71.2 ± 3.4%, and 95.6 ± 5.8% inhibition of tumor cell migration), respectively.

Fig. 5 Anti-invasive activity of METVAN against U87 glioblastoma and MDA-MB-231 breast cancer cells. Cells were incubated for 24 h with METVAN in concentrations ranging from 0.5 to 10 μM. The cells were then trypsinized and processed for invasion assays using Matrigel matrix-coated Boyden chambers. Data points, the mean (± SE) values from 3 independent experiments. The mean (± SE) IC_{50} values were 0.78 ± 0.4 μM and 0.61 ± 0.3 μM for the U87 and MDA-MB-231 cells, respectively.
Corps., Indianapolis, IN) as described earlier (27, 44). Cells were incubated with either METVAN in 0.1% DMSO or 1:16-diluted plasma samples from METVAN-treated mice for 48 h at 37°C, and were fixed, permeabilized, incubated with the reaction mixture containing TdT- and FITC-conjugated dUTP, and counterstained with propidium iodide. Cells were transferred to slides and viewed with a confocal laser scanning microscope (Bio-Rad MRC 1024) mounted on a Nikon Eclipse E800 series upright microscope as reported previously (27, 44).

**RESULTS AND DISCUSSION**

*In Vitro Activity of METVAN against Glioblastoma and Breast Cancer Cells.* The cytotoxic activity of METVAN against brain tumor and breast cancer cells was first confirmed using MTT assays. As shown in Fig. 1, METVAN exhibited potent cytotoxicity against each of the five brain tumor and four breast cancer cell lines with nanomolar or low micromolar IC_{50} values. The METVAN-induced cell death was confirmed to be apoptotic using the TUNEL of exposed 3’-OH termini of DNA with dUTP-FITC. As shown in the confocal laser scanning microscopy images in Fig. 2, METVAN-treated U87 glioblastoma and MDA-MB-231 breast cancer cells, examined for dUTP-FITC incorporation (green fluorescence) and propidium iodide counterstaining (red fluorescence), exhibited many apoptotic yellow nuclei (superimposed green and red fluorescence) at 24 h after treatment.

During the multistep process of tissue invasion, tumor cells initially adhere to the ECM proteins via cell surface integrin receptors and then gain migratory capacity to enter the surrounding tissue. ECM proteins such as laminin, fibronectin, vitronectin and type IV collagen are thought to play an important role in tumor cell attachment and migration because these proteins have been found in the basal lamina that promote the adhesion and invasion of tumor cells in situ (45–52). To determine whether METVAN is capable of impairing the adhesion of cancer cells to ECM proteins, cells were incubated for 16 h with noncytotoxic concentrations of METVAN (the cytotoxic IC_{50} values for 16 h incubation were 16.3 ± 1.8 μM for U87 and 11.1 ± 1.8 μM for MDA-MB-231 (see Fig. 1C) and the integrin-mediated cancer cell adhesion was examined. As shown in Fig. 3, pretreatment of cells with noncytotoxic concentrations of METVAN inhibited the adhesion of U87 glioblastoma and MDA-MB-231 breast cancer cells to laminin-, fibronectin-, vitronectin-, and collagen-coated plates. The inhibition of glioblastoma cell adhesion was concentration-dependent with average IC_{50} values of 1.4 ± 0.1 μM, 1.8 ± 0.1 μM, 1.2 ± 0.2 μM for laminin, fibronectin, vitronectin and collagen, respectively. The corresponding IC_{50} values for the MDA-MB-231 breast cancer cells were 1.0 ± 0.1 μM, 1.0 ± 0.0 μM, 1.1 ± 0.1 μM, and 1.3 ± 0.1 μM, respectively.

Dissociation and migration are the initial steps for infiltration of tumor cells into the surrounding tissue (53–55). To determine whether METVAN is capable of preventing the migration of tumor cells, U373 glioblastoma multicellular spheroids were used. The spheroids were prepared by plating the cells over agar plates as described above. Spheroids measuring 259.2 ± 41.9 μm in diameter were incubated with METVAN or with DMSO on fibronectin-coated coverslips. As shown in Fig. 4, U373 glioblastoma cells incubated with DMSO rapidly migrated to a distance 745 ± 29 μm away from the spheroid. Treatment of the spheroids with METVAN inhibited tumor-cell migration in a concentration-dependent fashion with an IC_{50} value of <1 μM. Treatment with 1 μM, 2 μM, and 5 μM METVAN resulted in 58.3 ± 7.3% (migration distance of 311.1 ± 54.4 μm), 71.2 ± 3.4% (migration distance of 214.9 ± 25.7 μm), and 95.6 ± 5.8% (migration distance of 32.6 ± 8.4 μm) inhibition of tumor cell migration from the spheroids, respectively (Fig. 4).

### Table 1: Laboratory studies of METVAN toxicity in mice

<table>
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<th>Laboratory test</th>
<th>Vehicle (n = 20)</th>
<th>METVAN dose level (mg/kg)</th>
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<tr>
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<td>12.5 (n = 20)</td>
<td>25 (n = 20)</td>
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<tr>
<td>Hematology</td>
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<td>WBCc (× 10^3/liter)</td>
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<td>Hgb (g/dl)</td>
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<tr>
<td>Plt. ct. (× 10^3/liter)</td>
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<td>400 ± 59</td>
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<tr>
<td>K (mmol/liter)</td>
<td>3.6 ± 0.1</td>
<td>3.7 ± 0.1</td>
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<tr>
<td>Ca (mg/dl)</td>
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<td>8.7 ± 0.1</td>
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<tr>
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<td>6.1 ± 0.2</td>
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<tr>
<td>Creat. (mg/dl)</td>
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<td>Alb. (g/dl)</td>
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<td>1.6 ± 0.0</td>
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<td>Total protein (g/dl)</td>
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<td>ALT (IU/liter)</td>
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<td>Alk. Ptse. (IU/liter)</td>
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<td>LD-L (IU/liter)</td>
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<tr>
<td>CPK (IU/liter)</td>
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<td>682 ± 222</td>
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* WBC, white blood count; Hgb, hemoglobin; Plt. ct., platelet count; K, potassium; Ca, calcium, PO_4, phosphorous; Creat., creatinine; Alb., albumin; Bili., bilirubin; LD-L, lactate dehydrogenase-liver derived; ALT, alanine aminotransferase; Alk. Ptse., alkaline phosphatase; CPK, creatine phosphokinase.
Tumor invasion of the basement membrane is a crucial step in the complex multistage process that leads to metastatic spread. Tumor cells cross the basement membrane as they initially invade the lymphatic or vascular beds during dissemination and as they penetrate their target tissues (56–59). The Matrigel matrix, an artificial basement membrane composed of growth factors and several ECM components such as collagens, laminin, and proteoglycans, was used to examine the effect of METVAN on tumor cell invasion. To determine whether METVAN impairs cancer cell invasion through Matrigel, the cells were incubated with various noncytotoxic concentrations of the compound, and then the invasion was examined using MDA-MB-231 and U87 cells (Fig. 5). METVAN inhibited the invasion of tumor cells through Matrigel matrix in a concentration-dependent fashion with mean IC₅₀ values of 0.78 ± 0.4 μM and 0.6 ± 0.3 μM for U87 and MDA-MB-231 cells, respectively.

Taken together, these findings show that treatment with METVAN at concentrations >1 μM is associated with a nearly complete loss of the adhesive, migratory, and invasive properties of the treated tumor cell populations.

In Vivo Pharmacokinetic Features of METVAN after i.p. Administration. We first evaluated the toxicity of METVAN in mice to identify nontoxic dose levels for pharmacodynamic studies. Mice were treated with single i.p. bolus injections of METVAN at dose levels of 12.5 mg/kg (n = 40), 25 mg/kg (n = 40), or 50 mg/kg (n = 40). Twenty mice from each group were electively killed on days 7 and 30 after the administration of METVAN. METVAN was not toxic to mice at these dose levels. None of the 120 mice treated with i.p. bolus injections of METVAN in this dose range experienced side effects or died of toxicity (data not shown). No evidence of acute toxicity was evident from laboratory tests performed on the blood samples from mice killed on day 7 (Table 1). In particular, there was (a) no neutropenia, anemia, or thrombocytopenia suggestive of a hematological toxicity, (b) no increase in serum creatinine levels or an electrolyte imbalance suggestive of renal toxicity, (c) no elevation of liver enzymes or total bilirubin levels suggestive of hepatic toxicity, and (d) no CPK elevation suggestive of cardiac toxicity (Table 1). Similarly, no laboratory abnormalities suggestive of subacute toxicity were evident from analysis of blood samples obtained from mice killed on day 30. No histopathological lesions were found in the organs of METVAN-treated mice that were electively killed at day 7 or day 30.

We next sought to determine whether cytotoxic concentrations of METVAN are achievable in vivo at nontoxic dose levels. To this end, a single nontoxic 10 mg/kg bolus dose of METVAN was administered to mice i.p., and the plasma vanadium concentrations were determined at various time points.
after the injection using inductively coupled plasma atomic emission (ICP) spectroscopy. A two-compartment, first-order pharmacokinetic model was fit to the pharmacokinetic data (Fig. 6, A and B). The computer-fitted pharmacokinetic parameter values are shown in Fig. 6B. Therapeutic plasma concentrations $\geq 5 \mu M$, which are highly cytotoxic against human cancer cells, were rapidly achieved and maintained in mice for at least 24 h after i.p. bolus injection of a single 10-mg/kg nontoxic dose of METVAN. At this dose level, the maximum plasma METVAN concentration ($C_{\text{max}}$) was 37.0 $\mu M$, which was achieved with a $t_{\text{max}}$ of 21.4 min. Notably, 1:16-diluted plasma samples obtained from METVAN-treated mice 30 min after administration of the drug killed 85% of MDA-MB-231 human breast cancer (A, A’) and NALM-6 human leukemic (B, B’) cells in vitro. After a 48-h incubation, cells were examined for apoptotic changes using standard TUNEL/apoptosis assays, as described in “Materials and Methods.”

**Fig. 7** Anticancer activity of plasma samples from METVAN-treated mice. Plasma samples, obtained from vehicle-treated (A, B) and METVAN-treated (A’, B’) mice 30 min after administration of the drug, were diluted with PBS 1:16 and then tested for their cytotoxic activity against MDA-MB-231 human breast cancer (A, A’) and NALM-6 human leukemia (B, B’) cells in vitro. After a 48-h incubation, cells were examined for apoptotic changes using standard TUNEL/apoptosis assays, as described in “Materials and Methods.”

In summary, in accordance with its potent in vitro activity and favorable in vivo pharmacokinetics, METVAN exhibited significant antitumor activity and delayed tumor progression in CB.17 SCID mouse xenograft models of human glioblastoma and breast cancer. The favorable in vivo pharmacodynamic features and antitumor activity of METVAN warrants further...
development of this novel oxovanadium compound as a potential new anticancer agent. More preclinical work remains to be done to determine the optimal dose and schedule for METVAN. We are planning to evaluate the efficacy of METVAN in comparison with, as well as in combination with, standard anticancer drugs, including taxanes (e.g., Taxol, Taxotere), thymidylate synthase inhibitors anthracyclins (e.g., Etoposide), and alkylating agents (e.g., ifosfamid, cyclophosphamide).

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In Vivo Antitumor Activity of Bis(4,7-dimethyl-1,10-phenanthroline) Sulfatooxovanadium(IV) \{METVAN [VO(SO_4)(Me_2-Phen)_2]\}

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