Dopamine Increases the Efficacy of Anticancer Drugs in Breast and Colon Cancer Preclinical Models

Chandrani Sarkar,1,3 Debanjan Chakroborty,1,3 Uttio Roy Chowdhury,1 Partha Sarathi Dasgupta,1 and Sujit Basu2,3,4

Abstract

Purpose: Because neurotransmitter dopamine inhibits vascular permeability factor/vascular endothelial growth factor (VEGF)–induced angiogenesis and as anti-VEGF agents act synergistically with anticancer drugs, we therefore investigated whether dopamine can increase the efficacies of these drugs.

Experimental Design: The effect of dopamine was investigated in human breast cancer (MCF-7) and colon (HT29) cancer–bearing mice. Experimental groups received either dopamine or doxorubicin or dopamine plus doxorubicin in MCF-7 tumor-bearing mice, and either dopamine or 5-fluorouracil or dopamine plus 5-fluorouracil in HT29-bearing mice. Thereafter, tumor growth, angiogenesis, tumor cell apoptosis, life span, and the effect of dopamine on the growth and survival of tumor cells in vitro were determined. Finally, the effects of dopamine on tumor vascular permeability; on VEGF receptor-2, mitogen-activated protein kinase, and focal adhesion kinase phosphorylation; and also on the proliferation and migration of tumor endothelial cells were investigated.

Results: Dopamine, in combination with anticancer drugs, significantly inhibited tumor growth and increased the life span when compared with treatment with dopamine or anticancer drugs alone. Dopamine had no direct effects on the growth and survival of tumor cells. The antiangiogenic action of dopamine was mediated by inhibiting proliferation and migration of tumor endothelial cells through suppression of VEGF receptor-2, mitogen-activated protein kinase, and focal adhesion kinase phosphorylation.

Conclusion: Our study shows that dopamine significantly enhances the efficacies of commonly used anticancer drugs and also indicates that an inexpensive drug like dopamine, which is being extensively used in the clinics, might have a role as an antiangiogenic agent for the treatment of breast and colon cancer.

The importance of angiogenesis as an integral component of tumor growth has fueled considerable efforts to develop efficient treatment regimens consisting of antiangiogenic agents that may inhibit the formation of new blood vessels in tumors and thereby the growth of these tumors (1–3). Angiogenesis inhibitors are considered to be relatively nontoxic and also these antiangiogenic agents exhibit different mechanisms of tumor growth inhibition than conventional anticancer agents (2–4). A major issue in oncology at present is the development of effective adjuvant therapy following resection of primary tumors to inhibit the growth of cancer cells remaining either locally or at remote sites and having the potential to grow into a recurrent tumor mass. Antiangiogenic therapy could restrain such lesion or even drive those cancer cells to apoptosis (1–6). Therefore, administration of antiangiogenic drugs in combination with anticancer drugs may be a better therapeutic strategy due to significant tumor growth–retarding property of these antiangiogenic agents (5, 6).

Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) is the prime cytokine reported to induce tumor angiogenesis by stimulating proliferation and migration of endothelial cells (1, 2, 7). VPF/VEGF is also reported to be expressed in most of the malignant tumors, and treatments that interfere with VPF/VEGF functions have shown promising results (1, 2, 5–7). Also, angiogenesis inhibitors that interfere with the expression or signaling of VPF/VEGF on tumor endothelial cells are reported to enhance the survival of cancer patients when used in combination with conventional cytotoxic...
anticancer agents (5–7). However, due to emergence of resistance to the currently used antiangiogenic drugs, it is essential to identify newer and effective antiangiogenic agents for the treatment of cancer (5, 7).

Dopamine is a catecholamine neurotransmitter that we recently showed to inhibit VPF/VEGF–induced angiogenesis (8). We have also shown that endogenous dopamine could be a potent regulator of tumor angiogenesis and its subsequent growth (9). Furthermore, as there are now several reports which indicate that antiangiogenic agents act synergistically with conventional anticancer drugs and because this combination is more effective than antiangiogenic agents used alone (5–7), it is prudent to investigate the potential of dopamine as an antiangiogenic drug in combination with other anticancer drugs. Accordingly, in the present study, we determined the efficacy of dopamine either alone or in combination with conventional anticancer drugs like doxorubicin or 5-fluorouracil (5-FU) in xenotransplanted human breast tumor (MCF-7) and orthotopically implanted human colon cancer (HT29), respectively. Our results for the first time showed that dopamine alone was effective in retarding the growth of these tumors by inhibiting tumor angiogenesis and most importantly dopamine showed significant synergism with conventional anticancer drugs. Also, this combination schedule significantly increased the survival of the tumor-bearing animals when compared with treatment with a single drug.

Materials and Methods

Materials

Dopamine was obtained from Abbott Laboratories; doxorubicin was from Pharmacia; and 5-FU was from Cadila Pharmaceuticals. CD31 rat anti-mouse monoclonal antibody and biotinylated anti-rat secondary antibody were from BD PharMingen. Vectastain Elite ABC kit was from Vector Laboratories. Tumor TACS in situ antibody were from BD PharMingen. VEGFR-2, (VEGFR-2), CD31, and CD34 monoclonal antibodies for flow cytometry analysis were from BD Biosciences PharMingen. VEGF, phospho–mitogen-activated protein kinase (MAPK), and phospho–cytokeratin were purchased from the American Type Culture Collection. MCF-7 human breast cancer and HT29 human colon cancer cell lines were purchased from the American Type Culture Collection. MCF-7 cells were cultured in DMEM supplemented with 10% fetal bovine serum and HT29 cells were maintained in minimal essential medium supplemented with 10% fetal bovine serum. Adherent monolayer cultures were maintained on plastic dishes and incubated at 37°C in a mixture of 5% CO₂ and 95% air.

Tumor transplantation and dopamine treatment

MCF-7. The human breast cancer cell line MCF-7 (2 × 10⁶ cells) was established by s.c. injection into the right flank of female athymic nude mice. Briefly, tumor cells were detached by trypsinization, harvested by centrifugation, resuspended in serum-free medium (200 μL), and mixed with equal volume of cold Matrigel (10 mg/mL). Cells were injected s.c. into 4- to 6-week-old nude mice pretreated with 60-d release estradiol pellets (10). Tumor volume was measured twice weekly and animals with tumor volume > 100 mm³ were randomly divided into two main groups, A and B (Table 1). The animals within these two groups were further randomized to one of the four treatment groups, including vehicle, dopamine (50 mg/kg/d i.p. × 7 continuous days), doxorubicin (5 mg/kg/i.p./single dose), and dopamine (50 mg/kg/d i.p. × 7 continuous days) + doxorubicin (5 mg/kg/i.p./single dose) (Table 1). Group A animals were sacrificed on day 8, and group B animals were followed to death. All experiments, except the survival study (14 animals per group was used for the survival study), were done in triplicate.

HT29. To produce cecal tumors, HT29 cells were harvested from subconfluent cultures by a brief exposure to 0.25% trypsin and 0.02% EDTA. Thereafter, trypsinized cells were centrifuged, resuspended in medium containing 10% fetal bovine serum, and the cells were washed once in serum-free medium and resuspended in HBSS. Only suspensions consisting of single cells with >90% viability were used for injection of 1 × 10⁶ cells in 50 μL of HBSS into the cecal wall of 4- to 6-week-old nude mice (11). Briefly, athymic nude mice were anesthetized with ketamine/xylazine, placed on a warm heating pad, and the abdominal cavity was opened to expose the cecum. The cecum was exteriorized through a small abdominal incision, and thereafter closed to detect tumors in the cecum. Animals with visible HT29 tumors in the cecum were randomly divided into two main groups, A and B (Table 2). The animals within these two groups were further randomized to one of the four treatment groups, including vehicle, dopamine (50 mg/kg/d i.p. × 7 continuous days), 5-FU (20 mg/kg/d i.p. for 5 continuous days), and dopamine (50 mg/kg/d i.p. × 7 continuous days) + 5-FU (20 mg/kg/d i.p. for 5 continuous days) (Table 2). Group A animals were sacrificed on day 8 and group B animals were followed to death. All experiments, except the survival

Table 1. Treatment groups for nude mice bearing athymic human breast cancer (MCF-7)

<table>
<thead>
<tr>
<th>Follow-up group</th>
<th>Vehicle</th>
<th>DA</th>
<th>DXR</th>
<th>DA + DXR</th>
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<tbody>
<tr>
<td>A. Sacrifice on day 8</td>
<td>8</td>
<td>8</td>
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<td>B. Follow to death</td>
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Abbreviations: DA, dopamine; DXR, doxorubicin.

Table 2. Treatment groups for nude mice bearing orthotopically implanted human colon cancer (HT29)

<table>
<thead>
<tr>
<th>Follow-up group</th>
<th>Vehicle</th>
<th>DA</th>
<th>5-FU</th>
<th>DA + 5-FU</th>
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<tr>
<td>A. Sacrifice on day 8</td>
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Synergism between Dopamine and Anticancer Drugs
study (14 animals per group was used for the survival study), were done in triplicate.

**Tumor growth measurement**

For s.c. MCF-7 breast cancer, tumor growth was determined by caliper measurement of the largest diameter and its perpendicular using the formula, tumor volume (mm$^3$) = $0.5 \times a \times b^2$, where $a$ is the largest diameter and $b$ is its perpendicular (13). At the completion of treatment, group A animals were sacrificed and their tumor tissues were collected by dissection. Thereafter, a portion of these tumor tissues was formalin fixed and embedded in paraffin, and another portion was snap frozen in liquid nitrogen and stored at -80°C for immunohistochemistry (11).

Similarly, for orthotopic HT29 colon cancer, group A animals were sacrificed after completion of the treatment. Thereafter, their tumor tissues were collected by dissection and tumor growth was determined by weighing the tumor mass. Finally, a portion of these tumor tissues was formalin fixed and embedded in paraffin, and another portion was snap frozen in liquid nitrogen and stored at -80°C for immunohistochemistry (11).

**Immunohistochemistry**

Tumor tissue samples were fixed in 4% paraformaldehyde, rinsed in PBS, transferred to 30% sucrose in PBS at 4°C, and frozen in optimum cutting temperature compound. Immunohistochemistry was done on frozen tissue sections using rat anti-mouse monoclonal antibody against CD31 using the Vectastain Elite ABC kit following the manufacturer’s protocol. Sections were counterstained with hematoxylin. Microvessel density was quantitated by analyzing 10 random fields per section (9).

**In vivo tumor cell proliferation**

The cellular kinetics of the tumor specimens were determined by immunohistochemical staining of BrdUrd. One hour before sacrificing the animals, BrdUrd (20 mg/kg of body weight) was injected i.p. into each animal. Subsequently, the animals were sacrificed and tumors were collected by dissection and their tumor tissues were snap frozen in liquid nitrogen and stored at -80°C for immunohistochemistry.
were excised and fixed in buffered formalin and embedded in paraffin. Cross-sections of the tissues were cut at 4-µm thickness. Tissue sections were then immunostained by using BrdUrd labeling and detection kit. The percentage of stained cells was counted and expressed as labeling index (14).

**In vivo tumor cell apoptosis**

Tumor cell apoptosis was assessed by measuring DNA fragmentation in a standard terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) assay according to the instruction of the manufacturer (R&D Systems). The number of apoptotic cells was expressed as apoptotic index (15).

**Separation of tumor endothelial cells**

A suspension of tumor cells (MCF-7 and HT29) was made by passage of viable tissue through sieve and treatment with collagenase and DNase. The cells were washed and the RBC was lysed with PharoMys (BD PharMingen). The cell pellets were then resuspended in fluorescence-activated cell sorting (FACS) buffer (1× PBS + 1% bovine serum albumin), preblocked with an Fc block (CD16/32), and then incubated with phycoerythrin-conjugated anti-VEGFR-2 (1:100), CD31 (1:100), and CD34 (1:100). Tumor endothelial cells were collected by FACS (9).

**Immunoprecipitation and immunoblotting**

Cell lysates were immunoprecipitated with different antibodies (1:100 dilution, except phospho-MAPK antibody was used at 1:1,000 dilution), and immunocomplexes were captured on protein A–agarose beads. Samples were subjected to SDS-PAGE and then transferred to polyvinyl difluoride membranes and immunoblotted. Antibody-reactive bands were detected by enzyme-linked chemiluminescence (Pierce Biotechnology, Inc.) and quantified by laser densitometry (16).

**Vascular permeability assay**

After completion of treatment with dopamine, Evans blue dye (100 µL of a 1% solution in 0.9% NaCl) was injected i.v. into mice. After 30 min, the animals were euthanized, and the whole tumor was removed. Evans blue dye was extracted from the tumor by incubation with formamide for 5 d at room temperature, and the absorbance of extracted dye was measured at 620 nm (17).

**Cell proliferation and viability assay in vitro**

Tumor cells (2×10⁵) or tumor endothelial cells were seeded in 24-well plates, cultured for 2 d, serum starved (0.1% serum) for 24 h, and then treated with either dopamine (1.2 nmol/ml) and/or various dopamine receptor antagonists, followed 5 min later by addition of 10 ng/ml VPF/VEGF or basic fibroblast growth factor. After culturing for 20 h, 1 µCi [³H]thymidine was added to each well and 4 h later, cells were collected for measurement of trichloroacetic acid–precipitable radioactivity. Cell viability was determined using trypan blue dye exclusion or the MTT assay (8).

**Cell migration assay in vitro**

Serum-starved tumor endothelial cells were washed twice with PBS and incubated with 4 mL solution (0.2 mg/mL collagenase, 0.2 mg/mL soybean trypsin inhibitor, 1 mg/mL BSA, 2 mmol/L EDTA in PBS) for 20 min at 37°C. Cells (1×10⁵ per well) were seeded into Vitrogen (30 µg/mL)–coated transwell chambers that were inserted into 24-well plates containing 1 mL of the same medium. After incubation at 37°C for 1 h, dopamine or dopamine D₂ receptor antagonists were added. VPF/VEGF was then added to a final concentration of 10 ng/mL. Following incubation for 2 h, the transwell membrane was stained with Cyquant DNA stain and migrating cells were counted in a spectrophotometer (8).

**Statistics**

The Kaplan-Meier method was used to determine the estimated survival curves and comparisons between the curves were done by log-rank test. Mean and SE were calculated. Differences among groups were evaluated by ANOVA and the unpaired Student’s test or Dunn’s multiple comparison tests. *P < 0.05* was considered significant (8, 18).

**Results**

**Effect of dopamine or doxorubicin or dopamine + doxorubicin on growth and survival of human breast cancer (MCF-7) in nude mice.**

The treatment started when the tumor volume in the MCF-7–bearing mice was ~100 mm³. At the end of the treatment, the tumor volumes in the vehicle, dopamine, doxorubicin, and dopamine + doxorubicin–treated animals were 413, 171, 133, and 63 mm³, respectively. Therefore, the tumor volumes in the vehicle-, dopamine-, and doxorubicin-treated animals were 413%, 171%, and 133%, respectively, of the original size (~100 mm³). In contrast, the tumor volume in the dopamine + doxorubicin–treated animals was only 63% of the original volume (100 mm³; Fig. 1A). Similarly, there was 24% increase in the life span of the animals treated with dopamine and 38% increase in the life span of the animals treated with doxorubicin when compared with vehicle-treated controls. However, striking increase in the life span (90%) was observed in animals treated with dopamine + doxorubicin.

![Graph](image-url)
(Fig. 1B) compared with the vehicle-treated controls. Also, 2 of 14 animals receiving combination of dopamine + doxorubicin showed tumor-free survival for the period of our experiments (until all the animals in the control groups died). It is to be noted here that treatment of the tumor-bearing animals with specific dopamine D₂ receptor antagonist domperidone before dopamine treatment abrogated the effect of dopamine, thereby indicating that the action of dopamine was through dopamine D₂ receptors (data not shown).

Effect of dopamine or 5-FU or dopamine + 5-FU on growth and 5-FU or dopamine + 5-FU on survival of human colon cancer (HT29) in nude mice. At the end of the treatment, dopamine treatment showed 58% decrease in tumor growth (Fig. 1C) and 30% increase in the life span when compared with vehicle-treated controls (Fig. 1D). Furthermore, 5-FU treatment alone caused 63% reduction in tumor growth (Fig. 1C) and there was also 38% increase in survival of the hosts in these 5-FU–treated animals compared with vehicle-treated controls (Fig. 1D). However, the combination treatment (dopamine + 5-FU) showed maximum inhibition of tumor growth (95%; Fig. 1C) and a marked increase in survival (97%) of these animals was noted compared with vehicle-treated controls (Fig. 1D). Also, 3 of the 14 animals receiving combination treatment showed disease-free survival throughout the period of experiments (until all the animals in the control groups died). It is to be noted here that treatment of the tumor-bearing animals with specific dopamine D₂ receptor antagonist domperidone before dopamine treatment abrogated the effect of dopamine, thereby indicating that the action of dopamine was through dopamine D₂ receptors (data not shown).

Effect of dopamine on the proliferation and survival of tumor cells in vitro. Thereafter, to investigate whether dopamine had any direct effect on MCF-7 breast cancer or HT29 colon cancer cells, these cells were treated in vitro with dopamine at a concentration of 1.2 nmol/ml. This concentration of dopamine was particularly selected because the antiangiogenic dose of dopamine (50 mg/kg i.p.), which we had used in our in vivo experiments, raised the plasma dopamine level of mice to this level at 1 minute after dopamine administration and, most importantly, this increase in plasma dopamine level in mice is also comparable with the plasma level reached in human.
subjects treated with an i.v. infusion of dopaminergic dose of dopamine (15). It was observed that dopamine had no direct effects on the proliferation and survival of these cancer cells as determined by thymidine incorporation and MTT assay (Fig. 2).

**Effect of dopamine on tumor vascular permeability and microvessel density in vivo.** Recent reports indicate that anti–VPF/VEGF agents can increase the concentrations of conventional anticancer drugs in the tumor tissues by decreasing vascular permeability, which in turn increases the efficacies of these drugs (2, 19, 20). Therefore, the effects of dopamine on vascular permeability were determined to elucidate the mechanism of synergism of dopamine with conventional antineoplastic agents. The results indicated that dopamine significantly inhibited tumor vascular permeability ($P < 0.05$) in mice bearing breast or colon tumors (Fig. 3A). Furthermore, this decrease in tumor vascular permeability was also accompanied by significant inhibition of tumor angiogenesis as evident from marked reduction of microvessel density ($P < 0.05$) in the tumor tissues (Fig. 3B and C). The other two members of the catecholamine family, namely epinephrine and norepinephrine, had no synergistic effects with antineoplastic drugs (data not shown).

**Role of dopamine on apoptosis and effect of dopamine or doxorubicin or 5-FU and dopamine + doxorubicin or dopamine + 5-FU on proliferation of tumor cells in vivo.** In addition to inhibition of tumor angiogenesis, dopamine treatment showed considerable increase in number of tumor apoptotic cells in both MCF-7 and HT29 tumors (Fig. 4A and B). Dopamine also caused significant inhibition in proliferation of the tumor cells (Fig. 4C and D). Similar inhibition in tumor cell proliferation was also observed after treatment with doxorubicin or 5-FU alone (Fig. 4C and D). However, maximum inhibition in tumor cell proliferation was observed in the groups receiving dopamine + doxorubicin or dopamine + 5-FU (Fig. 4C and D).

**Dopamine inhibits vascular permeability and microvessel density by down-regulating VEGFR-2, FAK, and MAPK phosphorylation in tumor endothelial cells in vivo.** VPF/VEGF is the prime cytokine expressed by the majority of human tumors (1, 2, 7). It plays a critical role in inducing vascular permeability, a critical step in the process of tumor angiogenesis (1, 2). The molecular mechanism of this action of VPF/VEGF is mediated through phosphorylation of VEGFR-2 and subsequent phosphorylation of FAK in tumor endothelial cells (21, 22). VPF/VEGF–induced proliferation of endothelial cells is also an important function in the angiogenic cascade and is mainly mediated through the MAPK pathway (23–25). Accordingly, dopamine-mediated inhibition of VEGFR-2, FAK, and MAPK phosphorylation was investigated in tumor

![Fig. 4. Effect of dopamine alone or in combination (doxorubicin or 5-FU) on tumor cell apoptosis and on tumor cell proliferation in human breast – and colon cancer – bearing nude mice. A and B, treatment with dopamine caused significant increase in tumor cell apoptosis in MCF-7 and HT29-bearing mice, when compared with the vehicle-treated control. The highest numbers of apoptotic tumor cells were evident following dopamine + doxorubicin (DXR) and dopamine + 5-FU treatment and dopamine + 5-FU. Graphical representation shows the number of apoptotic cells in each experimental group. Apoptosis was determined by TUNEL assay and by BrdUrd incorporation in tumor cells. Graphs represent cell proliferation index of various experimental groups. Columns, mean of three separate experiments; bars, SE (n = 8 for each experimental group; *, $P < 0.05$).](image-url)
endothelial cells. Although angiogenesis was associated with phosphorylation of VEGFR-2, FAK, and MAPK (Fig. 5A and B), dopamine-mediated significant inhibition of tumor angiogenesis, on the contrary, was accompanied by significant suppression of VEGFR-2, FAK, and MAPK phosphorylation in tumor endothelial cells (Fig. 5A and B).

Dopamine specifically inhibits VPF/VEGF–mediated tumor endothelial cell proliferation and migration in vitro. The specific direct anti–VPF/VEGF or antiangiogenic action of dopamine was next confirmed because although 1.2 nmol/ml of dopamine significantly inhibited VPF/VEGF–mediated proliferation and migration of tumor endothelial cells in vitro, it failed to inhibit tumor endothelial cell proliferation induced by basic fibroblast growth factor (Fig. 6A and B). In addition, dopamine also suppressed VPF/VEGF–induced phosphorylation of VEGFR-2 in these cells (Fig. 6C). Dopamine acted specifically through its D<sub>2</sub> receptors present on the tumor endothelial cells because treatment of these cells with specific dopamine D<sub>2</sub> receptor antagonist, eticlopride, before the treatment with dopamine abrogated the action of dopamine (Fig. 6A-C).

**Discussion**

Taken together, these results for the first time show that low nontoxic dose of dopamine, which is comparable with the low dopaminergic dose used in humans (15, 26), inhibits the growth of human breast and colon cancer in preclinical models. In addition, there is a synergistic effect when dopamine is used in combination with common anticancer drugs and this combination therapy increases the life span of tumor-bearing animals.

Because both the human tumors used in our experiments induce VPF/VEGF–mediated tumor angiogenesis and growth (27, 28) and because dopamine has been reported to inhibit the actions of VPF/VEGF (8), it can be concluded that the antiangiogenic effect of dopamine is due to inhibition of the functions of VPF/VEGF. In addition, VPF/VEGF–induced microvascular hyperpermeability is a critical step in the process of tumor angiogenesis (1, 2, 29). It has been further reported that VPF/VEGF primarily mediates its action by acting mainly through VEGFR-2 receptors present on the endothelial cells (1, 2). Recent reports suggest that FAK is an important molecule in VPF/VEGF–mediated signaling pathway (21, 30–32) and its...
phosphorylation leads to increased vascular hyperpermeability (30, 31). In the present study, dopamine showed a unique property in inhibiting hyperpermeability of tumor microvessels and thereby angiogenesis in human breast and colon malignant tumors by suppressing phosphorylation of VEGFR-2 and FAK in tumor endothelial cells, the two critical components of VPF/VEGF signaling cascade. This, in turn, led to inhibition of tumor growth. It is to be noted here that there are now reports that indicate that targeting FAK could be an option for developing novel anticancer therapy (33–35).

Furthermore, proliferation and migration are other two important functional aspects of endothelial cells for the process of angiogenesis and VPF/VEGF–induced activation of MAPK is critical for these processes (23–25). In untreated tumor-bearing mice, MAPK phosphorylation was significantly increased in tumor endothelial cells, which correlated well with angiogenesis in tumor tissues. In contrast, treatment of tumor-bearing mice with dopamine significantly inhibited phosphorylation of MAPK in tumor endothelial cells and this was accompanied with significant inhibition of tumor angiogenesis. Although we had previously reported that endogenous dopamine regulates VPF/VEGF–induced phosphorylation of VEGFR-2, FAK, and MAPK in normal mesenteric endothelial cells (16), our present results indicating dopamine-mediated inhibition of VEGFR-2, FAK, and MAPK phosphorylation in tumor endothelial cells is significant because critical functional and genetic differences have been now been discovered between tumor and normal endothelial cells (36–41). Therefore, our study enables to dissect the detailed mechanism of antiangiogenic effect of dopamine on tumor endothelial cells.

Finally, in contrast to the presently available VPF/VEGF inhibitors in the clinics, the price of dopamine (a small molecule) or its specific D2 agonists are much cheaper. This, in turn, may be very important in perspective of health economics for using the limited funds allocated for the treatment of cancer (42, 43). In addition, although new serious toxicities are being reported with the use of presently available anti–VPF/VEGF agents (5, 7), dopamine is a well-established drug with known and manageable toxicity (44). Furthermore, recent clinical reports have indicated the therapeutic efficacy of dopamine or its D2-specific agonists in the treatment of ovarian hyperstimulation syndrome, a serious disorder that is many times fatal (45, 46). The pathogenesis of this disorder is due to VEGFR-2–mediated increase in vascular permeability. Dopamine or its D2 agonists have shown to cure ovarian hyperstimulation syndrome patients by inhibiting the function of VEGFR-2 (45, 46). Thus, our present preclinical data need to be translated rapidly to the clinics for the treatment of breast and colon cancer.

**Fig. 6.** Effect of dopamine on basic fibroblast growth factor (bFGF) and VEGF-induced tumor endothelial cell proliferation, migration, and VEGF-induced VEGFR-2 phosphorylation in vitro. A, effect of dopamine on proliferation of tumor endothelial cells isolated from human breast (MCF-7) and colon (HT29) tumors grown in nude mice and stimulated in vitro by basic fibroblast growth factor and VPF/VEGF. Basic fibroblast growth factor stimulation of TEC proliferation was unaffected by dopamine. VPF/VEGF strongly stimulated TEC proliferation (*, P < 0.05 versus untreated or treated with dopamine only). The stimulatory effect of VPF/VEGF was abrogated (*, P < 0.05) by dopamine. Eticlopride, a D2-specific antagonist, abrogated dopamine-mediated inhibition of VPF/VEGF–induced TEC proliferation. B, experiments paralleling those of A, but measuring TEC migration rather than proliferation. C, dopamine inhibited VPF/VEGF–induced VEGFR-2 phosphorylation. Eticlopride, a D2-specific antagonist, abrogated dopamine-mediated inhibition of VPF/VEGF– induced VEGFR-2 phosphorylation. Columns, mean of four separate experiments; bars, SE.
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