The Antiangiogenic Agent SU5416 Down-Regulates Phorbol Ester-Mediated Induction of Cyclooxygenase 2 Expression by Inhibiting Nicotinamide Adenine Dinucleotide Phosphate Oxidase Activity

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ABSTRACT

Increased expression of cyclooxygenase (COX) 2 and the production of PGs appear to provide a survival advantage to transformed cells through the inhibition of apoptosis, increased attachment to extracellular matrix, increased invasiveness and the stimulation of angiogenesis. The purpose of this study was to determine whether an angiogenic antagonist, SU5416, could inhibit endogenous and phorbol 12-myristate 13-acetate (PMA)-mediated induction of COX-2 expression. SU5416 (5 μM) inhibited endogenous as well as PMA-mediated induction of COX-2 expression when analyzed by immunoblot and Northern blot analysis. However, COX-1 expression remained unchanged under similar conditions. PMA is a potent inducer of reactive oxygen species that can play an important role during the induction of COX-2 expression. Our results demonstrated that PMA-mediated induction of COX-2 expression was found to be dependent on NADPH oxidase activity. An inhibitor of NADPH oxidase (diphenyleneiodonium chloride) blocked the PMA-mediated induction of COX-2 expression. The oxidase complex exhibited a temporal pattern of activation after exposure to PMA in which maximum activation was observed at 30 min after the addition of PMA. Activation of NADPH oxidase was also inhibited by SU5416, whereas an inhibitor of epidermal growth factor receptor signaling was unable to prevent the PMA-mediated induction of NADPH oxidase activity. When we blocked the PMA-mediated production of reactive oxygen species by blocking NADPH oxidase with SU5416, COX-2 expression and PGE2 synthesis were also inhibited. Our results suggest that inhibition of NADPH oxidase activity, blocking of COX-2 expression, and PGE2 synthesis may represent novel targets for SU5416.

INTRODUCTION

Angiogenesis is essential for tumor growth and spreading and is likely to be regulated by several growth factors. Among the polypeptides known to be angiogenic, VEGF is the first tumor-secreted factor discovered to be capable of increasing vascular permeability and promoting cell proliferation and migration (1). A dominant process discovered to be capable of increasing vascular permeability and the production of extracellular matrix proteins (5–9). PGH2 is a diverse group of autocrine and paracrine hormones that mediate many cellular and physiological processes such as cell proliferation, inflammatory and immune responses, bone development, wound healing, hemostasis, reproductive function, glomerular filtration, and the production of extracellular matrix proteins (5–9). PGH2 is an intermediate in the formation of PGs. Two PG synthases catalyze the formation of PGH2, from arachidonic acid, COX-1, and COX-2 (6, 9). Although both proteins display similar enzymatic activity, they are the products of separate genes (10, 11). COX-2 is up-regulated by various factors, including cytokines, growth factors, and tumor promoters (12). On the other hand, COX-1 is constitutively expressed in most tissues and is thought to serve in general housekeeping functions such as maintaining gastrointestinal mucosal integrity (7, 13).

COX-2 is overexpressed in a variety of different tumors, including colon, lung, pancreatic, prostate, and head and neck cancers. The overexpression of COX-2 has been most strongly associated with colorectal tumorigenesis and inhibition of this enzyme with nonsteroidal anti-inflammatory agents inhibits intestinal tumorigenesis in rodent models (14–17). It is also reported that 90% of NSCLCs have been shown to express COX-2 at a moderate to high level (18–20). Soslow et al. (19) reported COX-2 expression in 67% of squamous cell carcinomas. In stage I NSCLC, increased expression of COX-2 has been shown to correlate with shortened survival (21). These data suggest that COX-2 overexpression may enhance metastatic potential (18, 22). Up-regulation of COX-2 is associated with increased tumor cell invasiveness and migratory capacity (23). Recently, it has been reported that COX-2 inhibitors may inhibit tumor angiogenesis, reduce PG production by acting on several potential cell sources such as tumor cells, endothelial cells, and stromal reactive cells (24, 25). The possible induction of VEGF expression by PGE1 and PGE2 in a nonneoplastic cell system suggests a probable role of COX-2 in the expression of VEGF and in regulating tumor angiogenesis (26, 27). Therefore, up-regulation of COX-2 represents a potentially important therapeutic target in lung cancer.

We found that SU5416, a class of antiangiogenic compound, inhibits endogenous as well as phorbol ester-induced expression of COX-2 in human lung carcinoma cells. We show that the PMA-mediated induction of COX-2 expression is dependent on NADPH oxidase activity because an inhibitor of this oxidase, DPI, blocked the phorbol ester-mediated induction of COX-2 expression. This activation of NADPH oxidase was also inhibited by the antiangiogenic agent SU5416. When we blocked the phorbol ester-mediated production of superoxides by blocking NADPH oxidase with SU5416, PGE2 synthesis was also inhibited in these cells.

MATERIALS AND METHODS

Cell Culture. NCI-H460 human large cell carcinoma cells, which have been demonstrated to express the COX-2 enzyme constitutively (28), were
Obtained from American Type Culture Collection and routinely cultured in RPMI 1640 (Life Technologies, Inc., Rockville, MD) supplemented with 10% FBS, 50 units/ml penicillin, and 50 mg/ml streptomycin. The NSCLC cell line A549 and MDA MB 231 were also obtained from American Type Culture Collection and cultured in DMEM (Life Technologies, Inc.) supplemented with 10% FBS, 50 units/ml penicillin, and 50 mg/ml streptomycin.

**Immunoblot Analysis.** The cells were treated as indicated and were lysed at intervals using NP40 lysis buffer containing 50 mM Tris-Cl (pH 7.8), 150 mM NaCl, 1% NP40, 10 mM EDTA, and 10 mg/ml protease inhibitor mixture (Sigma), briefly sonicated and incubated in ice for 30 min. Lysates were clarified by centrifugation (12,000 rpm for 5 min), and 50 µg of lysates were resolved on 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes for 1 h. Membranes were incubated overnight with anti-COX-1 and -COX-2 antibodies (Santa Cruz Biotechnology). In some experiments, specific kinase inhibitors (Calbiochem) such as PD98059 (MEK inhibitor); SB203580 (p38 MAPK inhibitor) and LY294002 (PI3k inhibitor) were used to compare the efficacy of SU5416 (Sugen) in preventing the PMA-mediated induction of COX-2 expression. Cells were preincubated with the specific kinase inhibitors for 1 h before the addition of PMA. Resveratrol, an antioxidant, was also used to study the PMA-mediated COX-2 induction in H460 cells. Cell lysates were prepared as indicated, resolved on a 10% SDS-polyacrylamide gels, and transferred to nitrocellulose membranes to analyze COX-2 expression. The same membrane was probed with anti-β-actin monoclonal antibodies and provided as loading control. Membranes were developed by using enhanced chemiluminescence system (Amersham Pharmacia Biotech, Piscataway, NJ) and exposed to Hyperfilm (Amersham Pharmacia Biotech).

**Northern Blot Analysis.** Total RNA was isolated using Tri-reagent (MRC). RNA samples (20 µg) were separated on a formaldehyde-agarose gel and blotted onto a nitrocellulose membrane. The blot was hybridized at 42°C with the 0.9 kb fragment of human COX-2 labeled with [32P]dCTP a using Nick Translation System (Invitrogen). The same membrane was stripped and reprobed with cyclophilin A message (1B15) to demonstrate equality of loading.

**PG Measurement.** A total of 1 × 10^6 cells were plated in triplicates for each experiment and serum starved for 24 h. The serum-free cells were treated as indicated with PMA (50 ng/ml) and SU5416 (5 µM). After 3 h of incubation, serum-free RPMI with (+/-) 15 µM arachidonic acid was replaced and further incubated for 1 h. Conditioned medium was collected and the PGE2 in the medium was measured by using stable isotope dilution techniques using gas chromatography-negative ion chemical ionization mass spectrometry.

**Measurement of NADPH Oxidase Activity.** Cell membranes were isolated by differential centrifugation according to the method of Pagano et al. (29), which has been modified by Mohazzab and Wolin (30). In brief, isolated cells from six 75 cm² culture flasks were harvested with cell dissociation solution (PBS-EDTA), washed once with ice-cold Dulbecco’s PBS, and centrifuged for 5 min at 700 × g. Supernatant was discarded, and the pellet was resuspended in 2.5 ml of ice-cold Tris-sucrose buffer ([pH 7.1) 10 mM Trizma base, 340 mM sucrose, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, and 10 µg/ml protease inhibitor mixture (Sigma)] and sonicated by four 15-s bursts. The cellular homogenate was clarified by centrifugation at 1475 × g at 4°C for 15 min to remove nuclei and unbroken cells. The supernatant was then centrifuged at 30,000 × g at 4°C for 30 min. The pellet was discarded, and the supernatant was additionally centrifuged at 100,000 × g at 4°C for 75 min. The pellet was resuspended in 150 µl of Tris-sucrose buffer and stored at −80°C. To analyze the NADPH oxidase activity, we have generated a standard curve using the xanthine-xanthine oxidase-cytochrome c assay and found that 25.2 nmol of superoxide were produced/min/mg xanthine oxidase at 25°C (31, 32). We then standardized the xanthine-xanthine oxidase reaction with lucigenin and established a quantitative relationship between the cytochrome c assay and the lucigenin assay: 25.2 nmol of superoxide/min/mg protein were equal to 108,000 RLU/min/mg, as measured by a Monolight 3010 luminometer. The RLU/min/mg protein obtained from the xanthine-xanthine oxidase-lucigenin assay was found to be independent of the presence of SU5416. Similarly, SU5416 did not affect the activity measured by xanthine-xanthine oxidase-cytochrome c assay (550 nm). From these standardizations, we have clearly demonstrated that the addition of SU5416 had no effect on lucigenin. Generation of superoxides in the membrane preparation was then measured by chemiluminescence as follows: in a 500-µl reaction buffer ([pH 7.0), 1 mM EDTA, 150 mM sucrose, and 5 mM lucigenin], 15 µg of cell membrane protein, and 100 µM NADPH as substrate were added and incubated at 25°C for 5 min. In some experiments, DPI, SU5416, or EGFR inhibitor was added in the reaction mixture.

**Results.** To investigate whether SU5416 regulate COX-2 expression, we analyzed endogenous COX-2 expression in two NSCLC cell lines (H460 and A549) after exposure to SU5416. SU5416 is a specific VEGF-RII inhibitor (Flk-1/KDR). These two NSCLC lines A549 and H460 expressed a significant basal level of COX-2. Initially, we measured endogenous COX-2 expression (Fig. 1A). Exposure to SU5416 produced a dose-dependent decrease in endogenous COX-2 expression in H460 and A549 cells (Fig. 1, A and B). We also observed that SU5416 was unable to prevent EGFR phosphorylation in H460 cell line when exposed to EGF for the different time period (Fig. 1C). In this study, we have also analyzed the effect of SU5416 on COX-1 expression because this compound inhibited the endogenous expression of COX-2 (Fig. 1, A and B). The results demonstrated that the COX-1 expression in these cell lines remained unchanged under similar experimental condition as mentioned in Fig. 1D. These results clearly indicate that SU5416 was specifically inhibiting COX-2 expression in these lung carcinoma cells, whereas COX-1 expression was not affected under similar experimental conditions.

Phorbol ester is a strong inducer of COX-2 expression (33). However, the mechanism of PMA-induced COX-2 expression is unclear. In this study, we report that SU5416 significantly inhibited the PMA-mediated induction of COX-2 expression in human lung carcinoma cells (Fig. 2A). In this experiment, serum-starved H460 cells were pretreated with either SU5416 or DMSO for 1 h and then treated with PMA (50 ng/ml) for 2 h. Results from this study demonstrated that SU5416 significantly (>80%) inhibited PMA-mediated induction of COX-2 expression. We also analyzed the COX-1 expression in the presence of PMA with or without SU5416 and found it remained unchanged under similar experimental condition (Fig. 2A). To further elucidate the mechanism responsible for changes in the levels of COX-2 protein, steady-state levels of COX-2 mRNA were determined by using Northern Blotting analysis (Fig. 2B). Serum-starved cells were treated with PMA or PMA+SU5416 for 2 h. Total RNA was isolated and subjected to Northern blot analysis. The result demonstrated that SU5416 significantly (>90%) inhibited the PMA-mediated induction of COX-2 mRNA. The same membrane was hybridized with the cyclophilin A (1B15) provided as a loading control. The Northern blot data are consistent with our observation reported for the immunoblot analysis (Fig. 2B). We also observed a similar effect of SU5416 in the human breast cancer cell line MDA MB 231 (Fig. 2C). PMA significantly induced the COX-2 expression in the human breast cancer cell line and the addition of SU5416 abolished the induction of COX-2 expression (Fig. 2C). In the next experiment, we compared the efficacy of SU5416 as a potent inhibitor of COX-2 expression with several specific kinase inhibitors such as PD98059, SB203580, and LY294002, also known to inhibit COX-2 expression under induced conditions (34, 35). Western blot analysis was performed using serum-starved cells that were treated with kinase specific inhibitors or SU5416 for 1 h before the addition of PMA. Our results demonstrated that the potency of SU5416 as an inhibitor of PMA-induced COX-2 expression at 5 µM concentration was highly effective when compared with the other known specific kinase inhibitors that were used at a higher dosage (25 µM; Fig. 2D). Taken together, these results suggest that SU5416 is a potent inhibitor of endogenous as well as PMA-induced COX-2 expression. To determine whether increased COX-2 synthesis was associated with increased formation of PGs, we evalu-
ated PGE_2 release in response to PMA and PMA+SU5416 with or without arachidonic acid. We measured PGE_2 release into the medium 4 h after the addition of PMA or PMA+SU5416. Fig. 3 shows PGE_2 release (pg/ml) in the absence of arachidonic acid (Fig. 3A) or in the presence (ng/ml) of arachidonic acid supplementation (Fig. 3B). In both cases, induction of PGE_2 release was detected after the addition of PMA. PGE_2 levels were increased from 38 to 80 pg/ml (2.1-fold) over a 4 h period in the absence of arachidonic acid (Fig. 3A) and from
The untreated cells. Although, the addition of SU5416 along with serum-free cells were treated as indicated. In some cases, cells were pretreated SU5416 (5 μM) for 1 h before the treatment with PMA. After 3 h of incubation, media were replaced with serum-free RPMI containing 0 μM arachidonic acid (A) or 15 μM arachidonic acid (B) and additional incubated for 1 h. Conditioned medium was collected, and the PGE2 release in the medium was measured by using stable isotope dilution techniques using gas chromatography-mass spectrometry.

The results from these experiments demonstrated the down-regulation of COX-2 expression by SU5416. However, this inhibitory effect of SU5416 on COX-2 expression may not be a target driven phenomenon because (a) a significantly high dosage of SU5416 was required in this study compared with its effective concentration (40 nM); (b) immunoblot analysis was unable to detect the VEGF receptor Flk-1/KDR (data not shown); and (c) addition of exogenous VEGF failed to induce COX-2 expression in mouse endothelial cells (data not shown). However, despite using higher concentrations of this drug, we did not observe any cytotoxic effects of SU5416 in this study over the experimental interval of 48 h. We also observed the PMA-dependent induction of COX-2 expression in human umbilical vein endothelial cells. However, SU5416 was unable to prevent this induction COX-2 by PMA (data not shown). This suggests a different signaling pathway is involved in the PMA mediated induction of COX-2 other than NADPH oxidase in this cell line.

PMA is a potent inducer of NADPH oxidase activity, and this oxidase activity is considered as the major source of ROS (30, 36, 37). Phorbol ester-mediated induction of COX-2 expression was found to be dependent on the generation of ROS (38). In this experiment, we used DPI as an inhibitor of NADPH oxidase. DPI is frequently used to study the NADPH oxidase, and this compound is highly specific in preventing the NADPH oxidase activity (39, 40). However, in some studies, DPI was used as inhibitor of xanthine oxidase (41). We used DPI at 20 μM to inhibit the oxidase activity and in higher concentrations in the COX-2 inhibition study and did not observe any noticeable cytotoxicity at these concentrations. In the next study, we have demonstrated that the increasing dosage of DPI (25–100 μM) prevented the PMA-mediated induction of COX-2 expression in H460 cells (Fig. 4A, top panel, Lanes 3–5). However, in a similar experiment, preincubation with the similar dosage (25–100 μM) of allopurinol, an inhibitor of xanthine oxidase had no effect on the induction of COX-2 expression by phorbol ester (Fig. 4A, Lanes 6–8). These results indicate that the activation of NADPH oxidase was involved during PMA-mediated induction of COX-2 expression in H460 cells, and these results were consistent with the previously reported findings regarding the induction of COX-2 expression mediated by the generation of ROS (38, 42). Because COX-2 is an indicative marker of oxidative stress (43), we have studied the effect of an antioxidant, resveratrol, in the PMA-mediated induction of COX-2. In this experiment, H460 cells were preincubated with resveratrol (50 μM) for 1 h and then treated with PMA for 2 h. A significant inhibition of PMA-induced COX-2 expression was observed in the resveratrol-treated cells (Fig. 4A, bottom panel, Lane 4). Subbaramiah et al. (44) previously reported that in mammary epithelial cells, resveratrol inhibited PMA-induced COX-2 transcription and PGE2 production.

Next, we demonstrated a temporal pattern of phorbol ester-mediated activation of the NADPH oxidase complex (Fig. 4B). In this study, H460 cells were treated with PMA for 15, 30, and 60 min, and the 100,000 × g membrane fractions were prepared as mentioned in “Materials and Methods.” NADPH oxidase activity was estimated by measuring the production of superoxide by lucigenin chemiluminescence method using a standard curve generated by xanthine-xanthine oxidase-cytochrome c assay (31, 32). Furthermore, this activity was inhibited by superoxide dismutase (data not shown). Datta et al. (45) reported that increases in the production of ROS were detectable at 5 and 15 min after the addition of PMA. Our results showed that in these lung cancer cells, superoxide production could be detected at 15 min (38.5 nmol/min/mg). Maximum production of superoxide was observed at 30 min (80 nmol/min/mg) after the addition of PMA and decreased after 1 h (Fig. 4B). The maximum production of superoxides we observed was similar to as reported by Salgado et al. (46). Because the maximum oxidase activity was observed at 30 min after the addition of PMA, we selected the 100,000 × g membrane fraction isolated at 30 min for the next series of experiments.

SU5416 blocked PMA-mediated activation of the oxidase. In this study, we used DPI as an inhibitor of NADPH oxidase (Fig. 5A) along with SU5416 to demonstrate the inhibitory effect of this antiangiogenic compound on oxidase activity. Cells were exposed to PMA for 30 min, 100,000 × g membrane fractions were isolated and then the inhibitors were added to the isolated membranes. A 4.6-fold induction of superoxide production was observed when the cells were treated with PMA for 30 min (15.7–72.6 nmol/min/mg). DPI at 20 μM inhibited 88% of superoxide production (8.2 nmol/min/mg), whereas 92% of superoxide production (6.4 nmol/min/mg) was inhibited by SU5416 (5 μM). In another experiment, an inhibitor of EGFR pathway (OSI-774, 10 μM; Genentech) was used to see its effect on the oxidase activity.
activity. However, this EGFR inhibitor had no significant effect on the oxidase activity (Fig. 5A). In another study, we preincubated the H460 cells with SU5416 for 1 h before a 30-min PMA exposure (Fig. 5B). A total of 100,000 × g membrane fractions were isolated and oxidase activity measured. Our results clearly demonstrate that the treatment with SU5416 inhibited the 83% of the PMA-mediated generation of superoxide. We also observed that the addition of only SU5416 significantly inhibited the superoxide production when compared with control cells (19.0–3.3 nmol/min/mg). These results are also consistent with our immunoblot data where we have reported this compound inhibited the endogenous expression of COX-2 (Fig. 1A). These results demonstrated that SU5416 was highly effective in preventing the PMA-mediated generation of superoxides. Therefore, our results from these two experiments (Fig. 5, A and B) indicate that NADPH oxidase activity may act as a novel target for SU5416.

In another study, we have analyzed the effect of SU5416 in
IL-1β-mediated induction of COX-2 (Fig. 5C). In this study, serum-starved H460 cells were pretreated with SU5416 for 1 h and then treated with either IL-1β (10 ng/ml) or PMA (50 ng/ml) for 2 h. Significant induction of COX-2 expression was observed by IL-1β (Fig. 5C, Lane 3), and SU5416 also blocked the IL-1β-mediated expression of COX-2 (Fig. 5C, Lane 6). Maihofner et al. (47) reported that IL-1β-induced COX-2 expression was mediated through the activation of NF-κB. It is also reported that IL-1β-mediated activation of NF-κB requires NADPH oxidase activity (48). Recently, Gu et al. (49) reported that RelA, involved in IL-1β-mediated NF-κB activation, associated with NADPH oxidase adapter protein p47(phox). Therefore, IL-1β-mediated induction of COX-2 expression was inhibited by SU5416 is consistent with these reported studies because we have demonstrated the inhibition of NADPH oxidase activity by SU5416.

SU5416 exhibits an absorption maxima at 440 nm (Fig. 6, panel 2) and, thus, emits a yellow color when solubilized. We observed that the isolated membrane fractions were yellow in color. Therefore, we scanned membrane fractions obtained from untreated cells and cells exposed to SU5416 (5 μM) for 30 min (Fig. 6). Scanning the membrane fractions obtained from control cells treated with vehicle alone (DMSO) from 250 to 600 nm did not indicate the presence of a maxima at 440 nm (Fig. 6, panel 1). However, membrane fractions obtained from cells treated with SU5416 produced a maximum at 440 nm (Fig. 6, panel 3). These results suggest that SU5416 accumulates in membrane fractions, and the data are consistent with SU5416 inhibition of NADPH oxidase, preventing the generation of ROS and ultimately down-regulating the expression of COX-2 in lung cancer cells.

**DISCUSSION**

Angiogenesis is a dynamic and complex process involving the formation of new blood vessels from the established vasculature (50). In normal healthy adults, this occurs only in pregnancy and in wound healing. In cancer, neovascularization is essential for both primary as well as metastatic tumor growth, leading to the conclusion that the antiangiogenic therapy may provide a novel and useful addition to current antineoplastic approaches (50). In lung cancer, the extent of angiogenesis appears to be important for prognostic stage and survival in prospective and retrospective case series (51–54). A dominant process regulating angiogenesis is the interaction of VEGF with its receptor (VEGF-R; Ref. 2). One approach to the modulation of VEGF-mediated angiogenesis is to use antibodies against the VEGF protein itself or to the receptor. Another approach to the interruption of VEGF activity is through the inhibition of VEGF-receptor tyrosine kinase by small molecule compounds. One example of such a VEGF-receptor 2 tyrosine kinase inhibitor is the quinolone derivative SU5416 (55). SU5416 is a potent inhibitor of VEGF activity at the concentration of 50 μM. It has been reported that SU5416 inhibits VEGF-induced endothelial cell growth and/or survival (56, 57), Akt kinase (58), and activation of the transcription factor, NF-κB. Activation of ERK1/2, Akt, and NF-κB has been implicated in VEGF-induced endothelial cell growth and/or survival that leads to tumor angiogenesis (59–61). It has been reported that these signaling pathways were also activated during the induction of COX-2 expression under various conditions. Maihofner et al. (47) reported that the activation of NF-κB was required for the IL-1β-
mediated induction of COX-2. In this study, we observed the IL-1β-mediated induction of COX-2 expression was significantly blocked by SU5416 in H460 cells (Fig. 5C). It is also reported that NADPH oxidase activity is necessary for the activation of NF-κB (48). Taken together, our result is consistent with these reported studies as we have demonstrated NADPH oxidase as a target of SU5416.

Phorbol esters are one of the potent inducers of ROS through the activation of NADPH oxidase (45). We observed the time-dependent stimulation of oxidase activity after the addition of PMA (Fig. 4B) that could be inhibited by SU5416 (Fig. 5, A and B). Induction of COX-2 was stimulated by the activation of NADPH oxidase (38). We demonstrated that both DPI, a known inhibitor of NADPH oxidase, and SU5416 inhibited PMA-induced expression of COX-2. We observed that an inhibitor of EGFR, OSI-774, had no impact in preventing the activation of NADPH oxidase by PMA (Fig. 5A). On the other hand, SU5416 was unable to prevent the EGF-mediated phosphorylation of the EGFR receptor (Fig. 1C).

The requirement of NADPH oxidase activity for endothelial cell proliferation and migration may have important clinical implications because the growth of tumors is dependent on new blood vessel formation. Overexpression of COX-2 is important in stimulating the endothelial cell motility and tube formation by increased production of proangiogenic factors (22). Abid et al. (62) reported that endothelial cell proliferation and migration require NADPH oxidase activity in mediating angiogenesis. Ushio-Fukai et al. (63) also reported that NADPH oxidase-derived ROS are important in VEGF signaling and angiogenesis. They observed VEGF stimulates O2− production that is essential to KDR Tyr-phosphorylation, cell proliferation, and migration in human umbilical vein (63). In recent years, there has been an effort to develop novel antiangiogenesis strategies that inhibit tumor growth. The results of the present study suggest that the inhibitors of NADPH oxidase complex may serve as a novel target for antiangiogenic therapy. It may be advantageous and feasible to develop novel multifunctional agent that inhibit both Flk-1 and NADPH oxidase activities.

REFERENCES


12. Williams, C. S., Morham, S. G., Langenbach, R., Loftin, C. D., Tiano, H. F., Vouloumanos, N., et al. (62) reported that endothelial...


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