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STI-571 inhibits in vitro angiogenesis

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Abstract

Compounds that block angiogenesis are effective in the treatment of certain cancers and other angiogenesis-related diseases. Many of these compounds specifically target the rapidly proliferating and migrating endothelial cell. However, angiogenesis is a multi-faceted process involving heterotypic interactions between various cell types. For example, PDGFBB is an important cytokine secreted by endothelial cells that attracts smooth muscle cells to surround and stabilize a nascent vessel. Therefore, we hypothesized that STI-571, a tyrosine kinase inhibitor with PDGFβ receptor activity, would inhibit angiogenesis through an anti-migratory effect on smooth muscle cells. We demonstrate that STI-571 completely inhibits in vitro angiogenesis in fibrinogen-embedded mouse aorta. Furthermore, this angiostatic property was due mainly to an anti-migratory and anti-proliferative effect upon smooth muscle cells. These data suggest that STI-571, in addition to its efficacy in the treatment of certain cancers, may also prove to be clinically useful in diseases characterized by unregulated angiogenesis.

Keywords: STI-571; Angiogenesis; Aortic ring assay

In diseases such as cancer, arthritis, and diabetic retinopathy, unchecked angiogenesis contributes to disease progression. For example, it was recognized over 25 years ago that a tumor deprived of a dense capillary bed is severely growth limited [1]. Therefore, numerous approaches have been instigated to limit angiogenesis at one or more of the critical stages including endothelial cell proliferation, migration, and adhesion, degradation of extra-cellular matrix, endothelial cell tube formation, and smooth muscle cell recruitment. Many of these approaches involve the use of specific inhibitors of endothelial cell proliferation, migration, and attachment. Peptide [2], protein [3,4], and synthetically derived molecules [5] have proven to be effective by various mechanisms. For example, the VEGF receptor tyrosine kinase inhibitor SU5416 inhibits in vitro and in vivo angiogenesis by inhibiting endothelial growth and migration [6].

STI-571 is a tyrosine kinase inhibitor with c-kit, c-Abl, and PDGFβ receptor activity [7–9]. It has also been shown to reduce Bcr–Abl mediated VEGF secretion [10]. The clinical results with STI-571 have been remarkable [11–13]. In chronic myelogenous leukemia (CML), STI-571 inhibits the kinase activity of the Bcr–Abl fusion protein resulting in selective loss of neoplastic hematopoietic cells [14]. In gastrointestinal stromal tumors (GIST), which harbor pathognomonic mutations in the c-kit gene STI-571 inhibits kit tyrosine kinase producing marked tumor regression [15]. By comparison with cytotoxic therapies STI-571 causes few systemic side effects.

In the study described in this report, the angiostatic effect of STI-571 was investigated using an in vitro model of angiogenesis. Previous studies have identified PDGFBB, the ligand for PDGFβ receptor, as a major growth factor involved in smooth muscle cell recruitment during angiogenesis [8,9]. Furthermore, inhibition of the PDGF signal transduction pathway by a PDGF receptor tyrosine kinase inhibitor blocked smooth muscle cell growth and migration in a rat model of

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restenosis [16]. Therefore, we hypothesized that STI-571, a tyrosine kinase inhibitor that acts on the PDGFβ receptor, might function indirectly as an angiogenesis inhibitor. To our knowledge, this is the first investigation of STI-571 as an angiostatic agent.

Materials and methods

Materials. STI-571 was a gift from Novartis (Basel, Switzerland). Bovine fibrinogen, ε-amino-*n*-caproic acid, protease inhibitor cocktail, mitomycin C, and thrombin were purchased from Sigma (St. Louis, MO). Protein A–Sepharose was purchased from Upstate (Waltham, MA). Recombinant bFGF was purchased from R&D (Minneapolis, MN) and recombinant PDGFBB was purchased from Biosource International (Camarillo, CA). The polyclonal PDGFβ receptor antibody was purchased from Upstate and the anti-phosphotyrosine antibody was from Auspep. Goat anti-rabbit and goat anti-mouse horse-radish peroxidase (GAR-HRP and GAM-HRP) were purchased from Chemicon (Temecula, CA). Enhanced chemiluminescence substrate (ECL) was purchased from Pierce (Rockford, IL). The PVDF membrane was from Millipore and polycarbonate PVDF for the migration assay was from Poretics (Livermore, CA). Diff Quick was from Baxter Scientific (McGraw Park, IL).

Cell culture and media. The cells used in this study were a generous gift from Professor Mike Hill, Royal Melbourne Institute of Technology, Bundoora, Vic., Australia. All primary cells were maintained in a humidified 37 °C incubator with 5% CO_2. Rat aortic smooth muscle cells (RASMC) were cultured in MEM containing 10% fetal bovine serum (FBS) and L-glutamine. Bovine aortic endothelial cells (BAEC) were cultured in α MEM containing 10% FBS and 3 ng/ml basic fibroblast growth factor.

Immunoprecipitation and Western blotting. RASMC were serum starved in MEM containing 0.5% FBS for 24 h. Next, 1 μM STI-571 was added and the cells were incubated for 3 h at 37 °C. Following the incubation, the cells were stimulated with 20 ng/ml PDGFBB for 5 min, washed twice with ice-cold PBS, and lysed in IP buffer (1× PBS containing 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, and 100 mM sodium orthovanadate, pH 7.4, diluted 1:100 with a protease inhibitor cocktail just before use). The lysates were incubated on ice for 30 min and then clarified by centrifugation at 10,000*g* for 10 min. One milligram of cell lysate was then incubated with 1.5 μg of anti-PDGFβ receptor antibody for 2 h with rotation at 4 °C. The immune complexes were then captured with protein A–Sepharose for 1 h at 4 °C. The immunoprecipitates were washed three times with ice-cold IP buffer and re-suspended in 20 μl of 2× sample buffer (125 mM Tris, pH 6.8, 4% SDS, 25% glycerol, 4 mM EDTA, 5% β-mercaptoethanol, and 0.005% bromophenol blue). The samples were boiled for 5 min and then analyzed by 8% SDS–PAGE. Following electrophoresis and transfer to PVDF membranes, the blots were probed with an anti-phosphotyrosine antibody overnight at 4 °C followed by GAM-HRP secondary antibody for 1 h at room temperature. ECL detection was carried out according to the manufacturer's instructions. Finally, the blots were re-probed as above with an anti-PDGFβ receptor antibody followed by a GAR-HRP secondary antibody.

Mouse aortic ring assay. For preparation of fibrin matrices, a 3 mg/ml solution of fibrinogen containing 0.25% ε-amino-*n*-caproic acid (in α MEM) was incubated on ice for 10 min. Two hundred and fifty microliters of this solution was then mixed with 1 U of thrombin and quickly pipetted into the wells of a 48-well plate. The mixture was then incubated at 37 °C for 30 min. During this incubation period, a male balb-c mouse 4–6 weeks of age was sacrificed and the thoracic aorta was dissected and placed in ice-cold α MEM. The aorta was flushed of any remaining blood and the fibroadipose tissue and co-lateral vessels were carefully removed. Using a scalpel blade, the aorta was cut into rings 1 mm in length and placed on top of the previously prepared fibrin matrix. Following an additional incubation at 37 °C for 30 min, the aortas were covered with a second layer of fibrin matrix, incubated as above, and then covered with α MEM containing 0.5% FBS. Aortas were maintained at 37 °C and photographs were taken beginning on day 3 of the experiment. The numbers of sprouting vessels were quantified using AIS image analysis software (Imaging Research).

Cell growth assay. BAEC or RASMC were plated in 24-well plates in triplicate at 10,000 cells/well. STI-571 was added at different concentrations and the cells were incubated for a further 72 h at 37 °C. After the incubation period, the cells were dispersed in trypsin and then counted with a Coulter counter.

Flow cytometry. For flow cytometry experiments, BAEC or RASMC were treated with either SCF or PDGFBB in the presence or absence of STI-571 as before. Cells were then dispersed in trypsin, washed with PBS containing 0.1% FBS, and then fixed in 80% ethanol for 20 min on ice. Cells were then washed twice with PBS/0.1% FBS and re-suspended in a PBS/0.2% Triton X-100 solution containing RNAse (1 mg/ml) and propidium iodide (50 μg/ml, PI). Samples were incubated in the dark at room temperature for 20 min. Finally, each sample was subjected to fluorescence activated cell sorter (FACS) analysis.

Wound healing migration assay. RASMC were grown to 85–90% confluence (in six-well plates) and then made quiescent by incubating with MEM containing 1% BSA. The next day, using a pipette tip, cells were scraped from the culture dish leaving a void space. The cells were washed twice with PBS and fresh MEM with 1% BSA was added. At this time, 10 ng/ml PDGFBB with 1 μM STI-571 was added and the cells were incubated for an additional 48 h. After the incubation, the cells were washed with PBS, stained with crystal violet for 5 min, and then photographed.

Micro-chemotaxis assay. The micro-chemotaxis assay has been described previously [17]. RASMC were dispersed in trypsin and washed twice with PBS before re-suspending in 1 ml MEM containing 0.1% BSA and then allowed to recover for 30 min at 37 °C. Next, the cells were treated while in suspension with different concentrations of STI-571 for 30 min at 25 °C. PDGFBB (10 ng/ml) was placed in the bottom wells of a chemotaxis chamber in triplicate and then covered with a collagen-coated (20 μg/ml) polycarbonate PVDF membrane with 8 μM pore size. The top half of the chamber was then placed onto the bottom half and 25,000 cells were added per well. The chamber was then placed in a humidified incubator for 3 h at 37 °C. Following the incubation, the membrane was stained with Diff Quick and the number of cells that had migrated through the membrane were counted in four random fields.

Statistics. A two-tailed *t* test (Microsoft Excel) or ANOVA (Minitab) was performed to determine whether results were statistically significant. The level of protection against a type I error was set at $\alpha = 0.05$. Where noted, statistically significant results are indicated by an asterisk (*).

Results

Inhibition of PDGFβ receptor phosphorylation in the presence of STI-571

STI-571 is known to inhibit c-kit, c-Abl, and PDGFβ receptor phosphorylation at an IC50 of about 0.1 μM [15]. A previous study has demonstrated that in 5A cells, STI-571 inhibits PDGFβ receptor phosphorylation at a dose of 1 μM within 3 h [9]. In the present study, serum starved RASMC were treated with 1 μM STI-571 and then stimulated with 20 ng/ml PDGFBB for 5 min. The cell lysates were immunoprecipitated with anti-PDGFβ receptor antibody and subjected to Western blotting

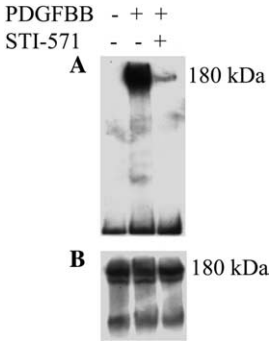

Fig. 1. Inhibition of PDGFβ receptor phosphorylation in the presence of STI-571. Serum starved RASMC were pre-treated with 1 μM STI-571 for 3 h and then stimulated with 20 ng/ml PDGFBB for 5 min. The cells were harvested and prepared for immunoprecipitation and Western blotting as described under Materials and methods. (A) Blots were probed with anti-phosphotyrosine antibody and (B) the same blot was re-probed with anti-PDGFβ receptor antibody. The arrows point to the PDGFβ receptor which migrates with a molecular mass of about 180 kDa.

with an anti-phosphotyrosine antibody. The results clearly demonstrate almost complete inhibition of PDGFβ receptor phosphorylation within 3 h of treatment with STI-571 (Fig. 1). A similar result was obtained with bovine retinal micro-vascular smooth muscle cells (data not shown).

Mouse aortic ring assay in the presence of STI-571

Fig. 2A shows representative mouse aortas treated without (a and b) or with (c and d) 1 μM STI-571 for 48 h. Treatment with STI-571 resulted in stasis, but not regression of vessels compared to control. In Fig. 2B, sections of mouse aorta were treated with either 0.1, 1, or 10 μM STI-571 for 48 h and the results were quantified using AIS image analysis software. In the figure, a clear dose–response is seen between 0.1 and 1 μM STI-571. The mean percent increase in vessel number was 58% of control at the 0.1 μM STI-571 dose and 26% of control at the 1 μM dose. The 10 μM dose caused cell rounding and complete disruption of vessel architecture (not shown). In Fig. 3, mouse aortas were incubated for four days and then treated with 1 μM STI-571. The aortas were photographed each day for an additional five days and the number of vessels was quantified with AIS software. Treatment with STI-571 at a non-toxic dose resulted in almost complete vessel stasis. Between days 4 and 9, the number of vessels in the control aortas increased between four- and fivefold while vessels in the STI-treated aortas increased less than onefold. The results of these experiments suggest that STI-571 inhibits new vessel growth in fibrinogen-embedded rat aortas but does not cause regression of existing vascular structures.

BAEC and RASMC growth in the presence of STI-571

The growth response of each vascular cell type to STI-571 was investigated. In the first set of experiments, subconfluent BAEC and RASMC were treated with 0–1 μM STI-571 for 72 h in their normal growth media. The cells were then dispersed in trypsin and counted. In

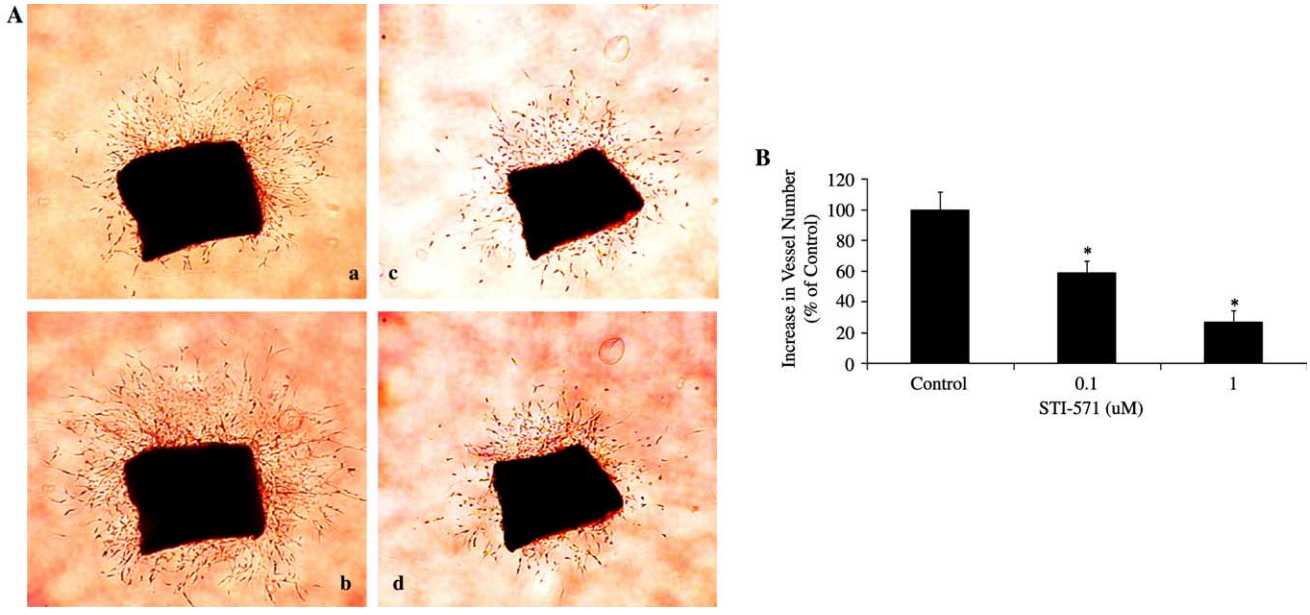

Fig. 2. (A) A photograph of mouse aortic rings assayed in the presence or absence of STI-571. (a) The control aorta photographed at day 0 of the experiment and again at day 2 (b). (c) The 1 μM STI-571-treated aorta photographed at day 0 and again at day 2 (d). (B) The dose–response assessment for STI-571 in mouse aortic rings. Fibrinogen-embedded mouse aortas were treated with different concentrations of STI-571 and incubated for an additional 72 h. Photographs were taken and the number of vessels was quantified using AIS image analysis software. Statistically significant results are indicated by an asterisk (*), $p < 0.05$, $n = 3$. The error bars are standard error of the mean (SEM).

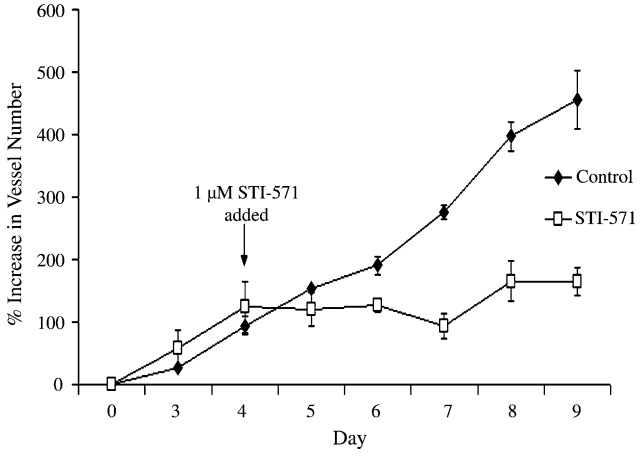

Fig. 3. Time course analysis of STI-571 in mouse aortic rings. Mouse aortas were incubated for four days and then 1 μM STI-571 was added. Photographs were taken each additional day and the number of vessels was quantified using AIS image analysis software, $n = 3$. The error bars are SEM.

multiple experiments STI-571 had no effect on BAEC cell numbers at doses up to 1 μM over a 72-h period. Similarly, RASMC cell numbers were not dose-dependently affected over a range of STI-571 concentrations but slight (20%) growth inhibition was observed at the 1 μM dose (Fig. 4A).

In the next set of experiments, BAEC or RASMC were grown in media containing reduced serum or BSA (0.5% FBS for BAEC and 1% BSA for RASMC) and then stimulated with 50 ng/ml stem cell factor (SCF) or 50 ng/ml PDGFBB with or without 1 μM STI-571 for 72 h. In BAEC, treatment with STI-571 alone reduced cell numbers by approximately 15–20% of control. Treatment with PDGFBB alone had no effect on cell numbers in BAEC compared to control. Cell numbers were reduced by 15–20% in groups treated with PDGFBB and STI-571 compared to groups treated with PDGFBB alone. Treatment with SCF alone had no effect in BAEC and the combined treatment with SCF and STI-571 reduced cell numbers by approximately 25% compared with the SCF only treated group (Fig. 4B).

In 1% BSA, RASMC numbers were no different in the control- and STI-571-treated group. However, both PDGFBB and SCF stimulated cell numbers to 111% and 26% above control, respectively. Combined treatment with PDGFBB and STI-571 reduced cell numbers by 55% of the PDGFBB only treated group. Combined treatment with STI-571 and SCF reduced cell numbers by 25% of the group treated with SCF only (Fig. 4B).

To determine the mechanism by which STI-571 inhibited PDGFBB-stimulated RASMC growth, cells were treated as in Fig. 4B and subjected to flow cytometry for ploidy. It was found that STI-571 alone reduced the number of cells in S-phase by approximately

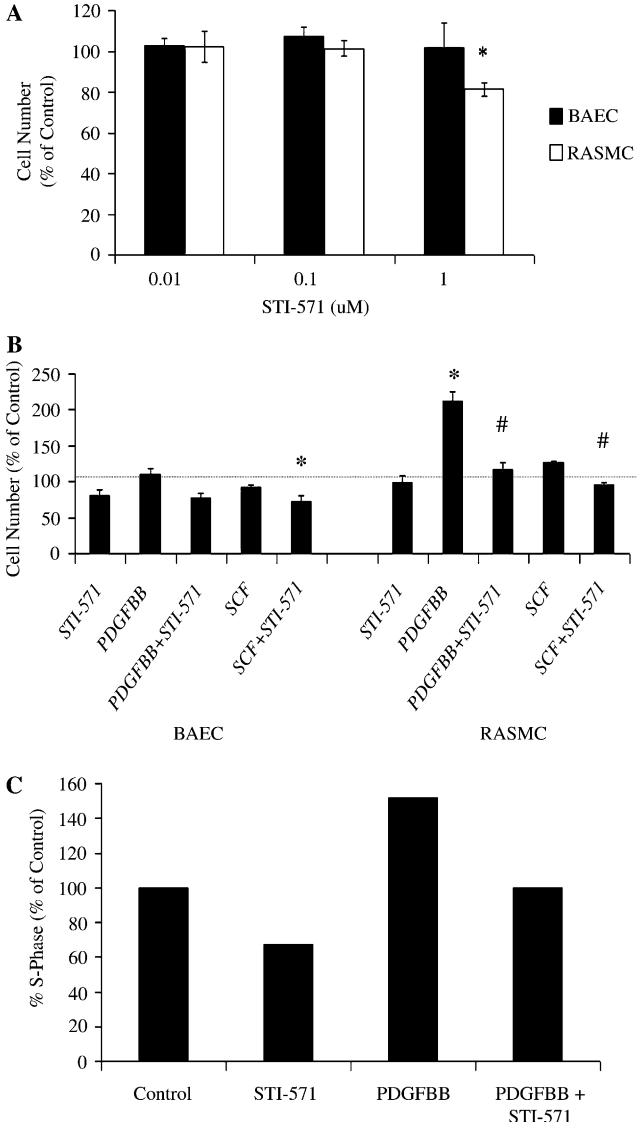

Fig. 4. Dose–response assessment for STI-571 in BAEC and RASMC cultures. (A) BAEC or RASMC in triplicate wells were treated with different concentrations of STI-571 (in media containing 10% FBS) and incubated for 72 h. The cell numbers were quantified by Coulter counter. Statistically significant results are indicated by an asterisk (*), $p < 0.05$. The error bars are SEM. (B) BAEC were serum starved in 0.5% FBS and RASMC were serum starved in 1% BSA followed by treatment with 1 μM STI-571 for 72 h +/- PDGFBB (50 ng/ml) or SCF (50 ng/ml). Cell numbers were then quantified by Coulter counter. (C) RASMC treated as indicated were fixed, stained with PI, and then subjected to flow cytometry for ploidy. Results are presented as percentage of cells in S-phase and as a percentage of control (set at 100%). * indicates that the results are statistically different from control and # indicates that the results are statistically different when comparing the group treated with cytokine only (PDGFBB or SCF) and the group treated with cytokine and STI-571 ($p < 0.05$).

a third while having no effect on apoptosis. Surprisingly, treatment with PDGFBB produced only a moderate increase in S-phase (approximately 50%), but this increase was completely abrogated by treatment with STI-571 (Fig. 4C). No detectable effect on cell cycle was

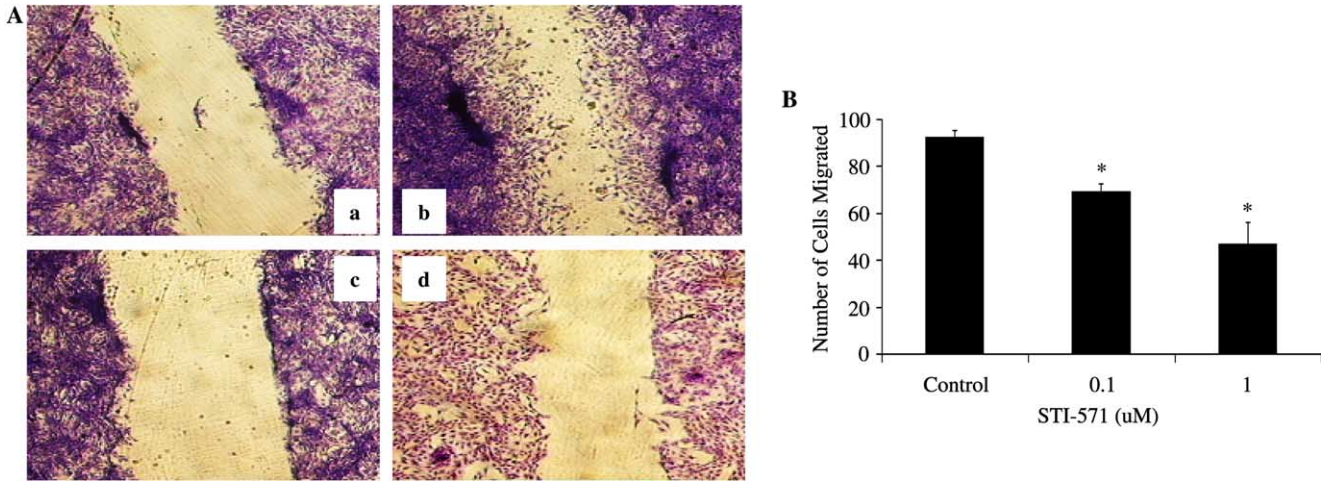

Fig. 5. RASMC migration in the presence of STI-571. (A) RASMC were made quiescent in MEM containing 0.1% BSA and treated with 1 μM STI-571 and 10 ng/ml PDGFBB. The migration of cells into space was then followed for 48 h before staining with crystal violet. The absence of PDGFBB (a) resulted in almost no migration while treatment with PDGFBB (b) resulted in invasion of cells into space. Treatment with PDGFBB and STI-571 (c) resulted in complete inhibition of migration. SCF did not cause migration of RASMC above background (d). (B) RASMC were treated with STI-571 for 30 min before loading into a chemotaxis chamber in triplicate. Migration was stimulated by 10 ng/ml PDGFBB. The number of migrating cells was counted and averaged in four random fields per well. Statistically significant results are indicated by an asterisk (*), $n = 3$. The error bars are SEM ($p < 0.05$).

observed in cells treated with SCF. Interestingly, the size (forward scatter) and internal complexity (side scatter) were strikingly changed by PDGFBB. This change in phenotype was reversed when the cells were treated with PDGFBB in addition to STI-571. No changes in cell cycle or apoptosis were detected in BAEC undergoing the same treatments outlined in Fig. 4B (data not shown).

RASMC migration in the presence of STI-571

To investigate the effect of STI-571 on RASMC migration two assays were utilized (Figs. 5A and B). In the first assay (A), RASMC migration was followed for 48 h in the presence or absence of PDGFBB and STI-571. In the absence of PDGFBB (a), virtually no background migration was observed. However, 10 ng/ml PDGFBB (b) stimulated migration was visible within 24 h as cells began to breach the leading edge of the monolayer. One micromolar of STI-571 (c) completely inhibited PDGFBB-stimulated cell migration after a 48-h incubation even in the presence of 100 ng/ml mitomycin C (mitomycin C is an inhibitor of proliferation and therefore isolates the effect of STI-571 on migration from proliferation, data not shown). One hundred nanograms per milliliter of SCF did not increase RASMC migration above background (d). In the next assay (B), a chemotaxis chamber was utilized. RASMC were pretreated with different concentrations of STI-571 for 30 min and then loaded into a chemotaxis chamber. RASMC migration towards 10 ng/ml PDGFBB was inhibited by 25% of control in the presence of 0.1 μM and by 50% of control in the presence of 1 μM STI-571. In separate experiments, STI-571 was shown not to inhibit the migration of BAEC in the presence of 10 ng/ml VEGF (data not shown). Taken together, these results suggest that STI-571 inhibits RASMC migration when stimulated by PDGFBB and that this effect is independent of inhibition of proliferation based on the results using the proliferation inhibitor, mitomycin C.

Discussion

In the present study, we reveal a novel role for STI-571 as an angiogenesis inhibitor. Previous studies have demonstrated that small sections of mouse or rat aortas can be cultured within a three-dimensional collagen or fibrin matrix and that de novo angiogenesis can be observed as vessel sprouts within three to four days [18]. These new vessels are comprised mainly of endothelial cells and smooth muscle cells that adopt a micro-vascular phenotype [18,19]. The addition of STI-571 completely blocked new vessel growth but did not affect existing vascular structures in fibrinogen-embedded mouse aorta. These results, in addition to knowledge that STI-571 inhibits PDGFβ receptor phosphorylation, led to the hypothesis that STI-571's angiostatic properties were related to an anti-migratory or anti-proliferative effect on smooth muscle cells.

In the later stages of angiogenesis, the importance of PDGFBB as a paracrine factor released by endothelial cells to recruit smooth muscle cells is confirmed by the gross vessel deformation in the PDGFβ receptor knockout mouse [20]. Other studies have shown that PDGFBB is strongly chemotactic for smooth muscle

cells in vitro [21]. In the present study, we clearly demonstrate STI-571's inhibitory effect on PDGFβ receptor phosphorylation in RASMC and an associated inhibition of migration in two separate assays. With only 30 min of treatment with 1 μM STI-571, migration was inhibited within the 3-h duration of the assay, thus excluding the effect of proliferation. In the wound healing migration assay, 1 μM STI-571 completely blocked RASMC migration in the presence of PDGFBB but not BAEC migration in the presence of VEGF even when proliferation was blocked with mitomycin C. Furthermore, SCF did not stimulate RASMC migration above background. These results suggest that STI-571's anti-migratory properties are specific to VSMC and may be limited to inhibition of PDGFβ receptor tyrosine kinase.

Although to our knowledge no prior studies have looked specifically at RASMC or BAEC growth in the presence of STI-571, it has been demonstrated that hepatic stellate cells [22] and rat mesangial cells [23] are dose-dependently growth inhibited by STI-571 when serum starved and stimulated with PDGFBB. As signaling through the PDGFβ receptor will activate intracellular downstream targets such as PKC α which is known to stimulate the growth and migration of VSMC [24,25], it was likely that at least some of STI-571's angiostatic properties were due to inhibition of SMC growth. Indeed, we demonstrated a moderate 20% growth inhibition of RASMC in the presence of STI-571 in 10% FBS. Surprisingly, RASMC growth was less inhibited by STI-571 alone when the cells were serum starved with 1% BSA. But when serum starved RASMC were treated with PDGFBB in addition to STI-571, a dramatic decrease in cell growth was observed compared to the PDGFBB only treated cells. This effect seems attributed to a decrease in S-phase following STI-571 treatment and not due to increased apoptosis. In BAEC, no change in BAEC numbers was seen when cells were treated with STI-571 in 10% FBS. However, a moderate decrease (15–20% of control) in cell number was seen when the cells were treated with STI-571 in 0.5% FBS in the presence or absence of PDGFBB. The purpose of these experiments was to isolate the effect of PDGFBB on either BAEC or RASMC growth and then determine what effect, if any, STI-571 had in the presence of the single growth factor. These results clearly demonstrate RASMC dependence on PDGFBB for growth and that blocking phosphorylation of PDGFβ receptor by STI-571 leads to growth inhibition. Furthermore, these results demonstrate that it is mainly the VSMC and probably not the endothelial cell that is STI-571's target.

The role of c-kit in RASMC or BAEC growth in the presence of STI-571 was determined by incubating each cell type with 50 ng/ml SCF for three days in reduced serum conditions. c-Kit is mainly expressed in hematopoietic progenitor cells, mast cells, and germ cells and is more critical during embryonic development than in adult homeostasis. However, c-kit and its ligand, SCF, are also found in vascular endothelium but it is unknown what role c-kit plays in processes related to angiogenesis [26,27]. In our hands, SCF moderately stimulated the growth of RASMC but had no effect in BAEC. The negligible effect of SCF in BAEC is consistent with a report by Broudy et al. [28] where they demonstrated no growth inhibitory or stimulatory effect in HUVEC treated with SCF (100 ng/ml) for seven days. Despite the slight growth stimulatory effect of SCF in RASMC, when the growth responses of SCF-stimulated and PDGFBB-stimulated RASMC are compared, it is clear that RASMC are much more dependent on PDGFBB for growth than SCF. This fact, coupled with our results showing no increase in RASMC migration above background when stimulated with a high concentration of SCF, suggests that STI-571's growth and migration-limiting properties appear to be due mainly to inhibition of PDGFβ receptor phosphorylation.

Finally, we cannot rule out the possibility that some of the growth and migration inhibitory effects of STI-571 in RASMC are due to direct inhibition of c-Abl phosphorylation. c-Abl is a non-receptor protein tyrosine kinase involved in cytoskeletal reorganization and is a downstream target of activated tyrosine kinases and Src family kinases [29]. Furthermore, c-Abl has been found to play a functional role in PDGFBB signaling mainly as an effector of cell ruffling and macropinocytosis which are processes that precede cell migration [30]. We found that both RASMC and BAEC express c-Abl (data not shown). Thus, it is possible that because c-Abl acts downstream of PDGFβ receptor, at least some of the anti-migratory or anti-growth properties of STI-571 are mediated by direct inhibition of c-Abl phosphorylation in RASMC. Although macro-vascular endothelial cells are not known to express high levels of PDGFβ receptor [31,32], it is unknown if c-Abl may act downstream of other growth factors involved in endothelial cell migration, namely VEGF and bFGF. However, we have demonstrated in this study that STI-571 did not adversely affect BAEC migration in the presence of VEGF nor did it substantially inhibit BAEC growth in 10% FBS. Experiments utilizing PDGFβ receptor blocking antibodies in addition to STI-571 should elucidate the functional role of c-Abl in SMC growth and migration.

In this study STI-571 was demonstrated to inhibit in vitro angiogenesis. We believe this angiostatic property is mainly due to an anti-migratory and anti-proliferative effect on smooth muscle cells, given the importance of PDGFBB signaling in smooth muscle cell migration and growth. Thus, it would appear that STI-571 may have direct action on tumors with a dysregulated PDGF signal transduction pathway [33] and also collateral

anti-angiogenic activity by interfering with vessel maturation as suggested by Ebos et al. [10]. Future studies may elaborate the anti-angiogenic activity of STI-571 in combination with KDR (VEGF receptor) tyrosine kinase inhibitors. Dual inhibition of PDGFβ receptor and KDR tyrosine kinase activity could be a promising therapeutic approach in diseases such as cancer where angiogenesis is an underlying factor [34].

Acknowledgments

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