STI571 Enhances the Therapeutic Index of Epothilone B by a Tumor-selective Increase of Drug Uptake

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ABSTRACT

Purpose: The purpose is to investigate whether STI571, through platelet-derived growth factor receptor inhibition, enhances the therapeutic response to the chemotherapeutic drug epothilone B (EPO906) and, if so, to analyze the mechanism(s) underlying the effect.

Experimental Design: SCID mice with s.c. human anaplastic thyroid carcinomas were treated with different doses of EPO906 alone or in combination with STI571 and with different timing of STI571 and EPO906 administration. Tumor growth, tumor interstitial fluid pressure (IFP), and uptake of EPO906 in tumors and normal organs were monitored.

Results: STI571 potentiated the therapeutic effect of EPO906. Tumors subjected to combination treatment were >40% smaller than those subjected to monotherapy with EPO906. The improved efficacy was matched by reduced tumor IFP and a 3-fold increase in the tumor levels of EPO906. No significant increase of EPO906 levels was seen in liver, kidney, or the intestinal tract. Cotreatment did not reduce the tolerability of EPO906, as determined by measuring body weight throughout treatment. STI571-induced reduction in tumor IFP and increase in tumor uptake required a minimum of three daily doses of STI571 and was not observed 3 days after last treatment with STI571. The enhancement of EPO906 therapeutic efficacy was only observed when STI571 was scheduled in a manner associated with reduced tumor IFP and increased tumor uptake of EPO906.

Conclusions: We conclude that STI571 increases the therapeutic index of EPO906 by selectively increasing the EPO906 uptake in tumors. The correlations between STI571 effects on tumor IFP and tumor drug uptake of EPO906 suggest a causal relationship between these phenomena. The study thus validates STI571 for combination treatment to enhance the therapeutic index of EPO906 in particular and, possibly, of chemotherapeutics in general.

INTRODUCTION

Interstitial hypertension is a feature of most solid tumors (1). The notion that increased IFP acts as a barrier for drug delivery into the tumor has recently found experimental support. Lowering of the IFP or, by other means, improving the transcapillary pressure gradient has in a number of studies been shown to augment the uptake of low molecular weight compounds, gases, and tumor-targeting antibodies (2–7). In some instances, it was further demonstrated that lowering of the tumor IFP and increased tumor uptake was paralleled by an enhanced effect of anticancer therapy (3, 6, 8). There is thus mounting evidence that the poor delivery of drugs from the bloodstream into the tumor interstitium can be augmented by adjuvant therapy with substances that lower the IFP.

PDGF is a mitogen for cells of mesenchymal origin, e.g., fibroblasts and smooth muscle cells, which acts by binding to two structurally related tyrosine kinase receptors (9). Different roles in the paracrine stimulation of tumor stroma have been described for PDGF. Firstly, PDGF promotes the formation of a rich stromal compartment, characterized by deposition of extracellular matrix components and blood vessel formation (10). Secondly, it was found that transfection of HaCaT cells with PDGF induced a tumorigenic phenotype (11). Thirdly, the desmoplastic response of human breast carcinoma is known to be initiated by PDGF (12). Finally, inhibition of PDGF was recently demonstrated to lower the tumor IFP in two different tumor models where PDGF receptor expression is restricted to the tumor stroma (5, 6). The reduction in tumor IFP was paralleled by an increased tumor uptake of paclitaxel (Taxol) and a concomitant enhancement of the therapeutic efficacy (6). PDGF receptor expression in tumor stroma occurs in a large fraction of common solid tumors (13). Large patient groups might therefore benefit from therapeutic targeting of this signaling pathway.

The tyrosine kinase inhibitor STI571 inhibits the kinase activities of the PDGF receptors, c-Kit, Abl, and ARG with similar potencies (14–16). STI571 is currently used in the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors, exploiting its inhibitory action on the Abl and c-Kit tyrosine kinases, respectively (17, 18). STI571 has also been used experimentally to demonstrate the involvement of PDGF signaling in the regulation of tumor IFP and trans-vascular transport (5, 6). In these preclinical studies, STI571 proved to potentiate the efficacy of coadministered chemotherapy by promoting an increased tumor uptake of the cytotoxic drug (6). Also, STI571 has recently shown therapeutic effects by inhibition of PDGF receptors in patients with
dermatofibrosarcoma protubers and chronic myelomonocytic leukemia (19, 20).

Epothilones constitute a new class of nontaxane microtubule stabilizing natural products (21, 22). One member of the epothilone family is EPO906, which has shown potent in vitro and in vivo antitumor activity. Compared with paclitaxel, several attractive features positively distinguish EPO906 from the taxanes, including its increased water solubility, as well as its activity against multidrug-resistant cells overexpressing P-glycoprotein or harboring tubulin mutations (21). EPO906 is currently undergoing Novartis-sponsored Phase II clinical trial in a variety of indications.

In the present preclinical study, we have investigated the effects of STI571 on tumor uptake and therapeutic index of EPO906 and also analyzed the effects of STI571 on EPO906 uptake in normal tissue. Furthermore, the kinetics of the STI571-induced effects on tumor IFP and tumor uptake of EPO906 have been characterized. Results from these analyses have been used to design therapeutic studies that investigate the correlations between the effects of STI571, on one hand, on the effects on tumor IFP and drug uptake, and on the other hand, on the therapeutic effects of EPO906.

MATERIALS AND METHODS

Materials. EPO906 and STI571 (CGP057148B) were provided by Novartis Pharma AG. The human anaplastic thyroid carcinoma cells KAT-4 cells were kindly provide by Dr. Kenneth B. Ain (Lexington, KY) and have been described previously (23).

Establishment of Tumors and Animal Care. Fox Chase SCID mice (ages 6–10 weeks; M&B, Ry, Denmark) were injected s.c. in the left flank with 2 × 10⁶ KAT-4 cells in a vehicle of 200 μl of PBS. The mice were monitored regularly according to United Kingdom Coordinating Committee on Cancer Research guidelines (24). All animal experiments described in this study were approved by the committee for animal experiments at Uppsala University.

Administration of Drugs. STI571 was administered at 100 mg × kg⁻¹ × day⁻¹ if not otherwise stated and given as a single oral dose in 200 μl of PBS. EPO906 was administered s.c. once weekly at a site distant from the tumor on the opposite side of the body and in 200 μl of vehicle consisting of 30% PEG-300 and 70% of a 0.9% NaCl solution. When STI571 and EPO906 were administered on the same day, STI571 dosing preceded EPO906 by 1–2 h.

Efficacy Studies. The experiments were started when the average tumor volume was ∼100 mm³. Tumor volume and body weight were subsequently recorded twice weekly. In addition to presenting changes in tumor volumes over the course of treatment, antitumor activity is expressed as percentage of T/C (mean increase of tumor volumes of treated animals divided by the mean increase of tumor volumes of control animals multiplied by 100). Tumor volume was calculated as \( V = a^2 \times \frac{b}{6} \), where \( a \) and \( b \) equal the short and the long diameter of the tumor, respectively. Mice were sacrificed if the body weight loss exceeded 20% or if the general condition of the mouse was poor. In all studies, EPO906 was administered on days 4, 11, and 18 after randomization. Except for the experiment in Fig. 1, which was concluded on day 18, all efficacy studies were concluded on day 21.

Pharmacokinetic Studies. EPO906 was administered on day 6 after randomization and the mice were sacrificed either 2 or 24 h after the administration of EPO906. Animals sacrificed 24 h after administration of EPO906 received an additional dose of vehicle or STI571 1–2 h before harvest. At harvest, the mice were anesthetized, blood was sampled by heart puncture, and after cervical dislocation, the tumor, one kidney, one lobe of the liver, and the entire small intestine were excised and weighed. Blood samples were supplemented with 2 mM eserine (Sigma, Stockholm, Sweden) and with sodium citrate to prevent coagulation. Solid tissues were immersed in an equal volume (1 g = 1 ml) PBS containing 4 mM eserine. All samples were immediately snap frozen in liquid N₂ and stored at −70°C until analysis.

Analysis of the Content of EPO906 in Tissues and Blood. The concentrations of EPO906 in blood and tissues were determined using a specific liquid chromatography-tandem mass spectrometry method with APCI interface and positive ions detection. EPO906 was extracted by a liquid-liquid process with tert-butyl methyl ether in alkaline medium (35% sodium carbonate solution), including stable labeled EPO906 as an internal standard. Tissues (0.10–1.46 g) were homogenated in 2.0 ml of PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer [pH 7.4]) containing 3 mM eserine. Blood was treated with eserine at a final concentration of 2 mM at bleeding, for unknown samples, and before spiking in standard and quality control samples.

Measurement of Tumor IFP. Tumor IFP was measured by the wick-in-needle technique, as described previously (5, 25). Briefly, a standard 23-gauge needle, connected with a pressure transducer, was inserted into the central part of experimental tumors of anesthetized mice, and the pressure was monitored for a period of 10 min. For the experiment in Fig. 6b, the baseline tumor IFP of mice carrying large KAT-4 tumors (>1 cm³; \( n = 4 \)) was measured on day 1, and subsequently, treatment with STI571 was started. On day 3, 1–2 h after the last administration of STI571, tumor IFP was measured once again, and treatment was discontinued. Finally, on day 6, another measurement of...
tumor IFP in the same tumors was performed. For the experiment in Fig. 8a, mice received administration of control (n = 5) or STI571 (n = 5) on the day before and 1–2 h before the IFP measurement. For the experiment in Fig. 8c (n = 5 in each group), STI571 or vehicle was administered 48 h before (day 1), 16 h before (day 2), and 1–2 h before (day 3) the measurement of tumor IFP.

**Immunohistochemistry.** Tumor halves were fixed in phosphate-buffered formaldehyde (pH 7.4; J. T. Baker, Tombro, Göteborg, Sweden) for 24 h at 4°C and paraffinized using an automated tissue processor (TPCduo 15; Medite). Of each tumor half, 3-μm sections were cut and immunohistochemically stained as follows. Each staining was performed on one section from each of the tumors.

The fraction of mitotic cells in the sections was estimated by incubation with an antibody (MPM-2; Dako, Stockholm, Sweden) that recognizes a mitosis-specific phosphoepitope. Antigen retrieval with 1 mM EDTA (pH 8.0) for 10 min at 98°C was required before immunohistochemistry. To reduce background attributable to endogenous mouse IgG, the mouse anti-MPM-2 antibody was incubated with the Animal Research Kit (Dako) at a dilution of 1:50 at 4°C overnight. After incubation with streptavidin-horseradish peroxidase (Animal Research Kit; Dako), sections were incubated in freshly prepared aminoethylcarbazole (Sigma, Stockholm, Sweden) for 11 min and counterstained for 0.5 min in Mayer’s hematoxylin (Medite). Finally, coverslips were mounted using Aquatex (Merck, Stockholm, Sweden). For staining of apoptosis, sections were deparaffinized, immersed in a citrate buffer (pH 6.0), and boiled for 2 × 6 min at 750 W in a microwave oven. Apoptosis was visualized in epithelial cells using the M-30 CytoDEATH antibody (1:10; Roche), and the staining procedure was performed on a NexES immunostainer with a diaminobenzidine substrate kit (Ventana Medical Systems, Tucson, AZ). Staining of CD31+/ blood vessels was performed as described previously (26). Staining of desmin+ mural cells was performed according to the manufacturer’s instructions using the EPOS Desmin-HRP kit (Dako).

In each section, the number of positively staining cells in 10 randomly chosen fields of vision (MPM-2, 0.056 mm², ×400 magnification; apoptosis, CD31, and desmin, 0.09 mm², ×400 magnification) of viable tissue was quantified. All quantifications were performed in a blinded manner.

**In Vitro Growth Curves.** KAT-4 cells were cultured in RPMI 1640, supplemented with 10% fetal bovine serum and antibiotics. KAT-4 cells were seeded at a concentration of 3000 cells/well in a 96-well plate, and STI571 and/or EPO906 were added in various concentrations (STI571, 0–10 μM; EPO906, 0–1 nM) to wells in triplicate. Cell culture medium and drugs were replaced every day. After 72 h in culture, the proliferation of the cells was measured using a colorimetric one-solution assay (Promega), and the background from control wells containing no cells was subtracted.

**Statistical Analyses.** All statistical analyses were performed using the two-sided, unpaired Student’s t test with a P < 0.05 considered significant. All data are displayed as mean ± SE.

**RESULTS**

**Treatment with STI571 Potentiates EPO906 Antitumor Efficacy in the KAT-4 Tumor Model.** KAT-4 thyroid carcinoma tumors express PDGF receptors exclusively in the stroma compartment and respond to PDGF receptor inhibition by lowering of the IFP (6). To determine whether the growth of KAT-4 tumors was sensitive to EPO906, tumor-bearing mice were treated with vehicle or with different doses of EPO906. As seen in Fig. 1, treatment with EPO906 inhibited the growth of the s.c. KAT-4 thyroid carcinoma xenografts in a dose-dependent fashion. Calculation of the increase of tumor volumes of treated animals divided by the increase of tumor volume of control animals multiplied by 100 (%T/C), gave values of 55.1, 22.9, and −9.6, respectively, for the doses of 0.3, 1.0, and 3.0 mg/kg × week−1. As judged by body weight loss, the two highest doses of EPO906 were not well tolerated, and the mice receiving 3.0 mg/kg × week−1 had to be sacrificed before the end of the experiment (data not shown).

To investigate the effect of combination treatment with STI571, tumor-bearing mice were divided into groups receiving either vehicle, STI571 alone, 0.3 or 1 mg/kg × week−1 EPO906, or STI571 in combination with 0.3 mg/kg × week−1 EPO906. Treatment with STI571 alone did not affect tumor growth (Fig. 2a), in agreement with previous studies (6). Combination treatment with STI571 potentiated the antitumor effects of the moderately active dose of 0.3 mg/kg × week−1 EPO906. Thus, the inclusion of STI571 in the treatment with 0.3 mg/kg × week−1 of EPO906 caused a suppression of the tumor growth rate that resulted in final tumor volumes in the combination treatment group that were 42.7% smaller than those in the corresponding EPO906 monotherapy group (Fig. 2a). It is noteworthy that the combination of STI571 and 0.3 mg/kg × week−1 EPO906 produced efficacy similar to that seen with 1.0 mg/kg × week−1 EPO906 (Fig. 2a). In agreement with previous observations that PDGF receptor inhibition improves the efficacy of pacitaxel, these data demonstrate that STI571 potentiates the antitumor effects of EPO906.

To provide cell biological correlates of the antitumor effects of the different treatment regimens, the number of apoptotic and mitotic tumor cells was assessed. As shown in Fig. 2b, combination treatment with STI571 and EPO906 was associated with a significant increase in apoptotic cells as compared with the EPO906 monotherapy group, which, in turn, displayed more apoptosis than the vehicle treatment group. Consistent with the ability of microtubule-stabilizing agents to block cells in mitosis, an increase in MPN-2-positive tumor cells was observed after combination treatment (Fig. 2c). We thus conclude that the cotreatment with STI571 enhances the antitumor effects of EPO906.

**STI571 Increases the Tumor Uptake of EPO906 but Does Not Alter Blood Vessel Density, Pericyte Coverage, or the Sensitivity of KAT-4 Cells to EPO906.** To characterize the mechanism(s) underlying the enhanced antitumor effect of the combination therapy, we investigated the effects of STI571 on tumor uptake of EPO906, the effects of single or combination treatments on blood vessel density and pericyte coverage and the possibility that STI571 sensitized KAT-4 cells to EPO906.
Firstly, a pharmacokinetic study was performed to investigate the effects of STI571 on tumor levels of EPO906. Tumor-bearing mice were divided into size-matched groups that were treated with three daily doses of vehicle or STI571 before receiving 0.3 mg kg\(^{-1}\) EPO906. The mice were sacrificed either 2 or 24 h after the injection of EPO906, and the tumor content of EPO906 was determined by liquid chromatography-tandem mass spectrometry method analysis of tumor homogenates. Compared with the concentration of EPO906 in tumors of control animals, the EPO906 concentration in tumors of STI571-treated mice was 3.0- and 1.6-fold higher at the early and late time point, respectively (Fig. 3). Notably, the levels of EPO906 in the tumors from mice receiving the combination treatment with 0.3 mg kg\(^{-1}\) EPO906 and STI571 was similar to those in tumors from animals treated with 1.0 mg kg\(^{-1}\) EPO906 (data not shown).

Secondly, tumor angiogenesis was analyzed. Determination of vessel density did not reveal any significant differences between tumors from animals having received control treatment or treatment with either EPO906 alone or in combination with STI571 (Fig. 4a). Pericytes express PDGF receptors and are important for capillary function and integrity (27–29). CD31 structures were therefore characterized with regard to coverage by desmin-positive cells. However, no significant differences between the three treatment groups were observed (Fig. 4b), ruling out major differences in recruitment of mural cells as the cause for the observed effects.

Finally, the effect of STI571 on EPO906-induced growth inhibition of in vitro cultured KAT-4 tumor cells, which do not express PDGF receptors, was studied. The proliferation of KAT-4 tumor cells was monitored over the time of treatment. Treatment with STI571, administered once daily, started at day 1. EPO906 was administered once weekly beginning on day 4. * P < 0.05 versus 0.3 mg kg\(^{-1}\) × week\(^{-1}\) EPO906 alone (n = 6). a, KAT-4 tumor volume was monitored over the time of treatment. Treatment with STI571, administered once daily, started at day 1. EPO906 was administered once weekly beginning on day 4. * P < 0.05 versus 0.3 mg kg\(^{-1}\) × week\(^{-1}\) EPO906 alone (n = 6), or STI571 in combination with 0.3 mg kg\(^{-1}\) × week\(^{-1}\) EPO906 (n = 6). b, Number of apoptotic cells (b) and mpm-2-positive cells (c) were determined in 10 high power fields of sections from six tumors, excised at the end of treatment, of each group. ** P < 0.01 versus EPO906 monotreatment; *** P < 0.001 versus vehicle.

**Fig. 2** STI571 potentiates the efficacy of EPO906. Tumor-bearing mice were treated with vehicle (n = 6), STI571 alone (n = 6), 0.3 mg × kg\(^{-1}\) × week\(^{-1}\) EPO906 alone (n = 7), 1.0 mg × kg\(^{-1}\) × week\(^{-1}\) EPO906 alone (n = 6), or STI571 in combination with 0.3 mg × kg\(^{-1}\) × week\(^{-1}\) EPO906 (n = 6). a, KAT-4 tumor volume was monitored over the time of treatment. Treatment with STI571, administered once daily, started at day 1. EPO906 was administered once weekly beginning on day 4. * P < 0.05 versus 0.3 mg × kg\(^{-1}\) × week\(^{-1}\) EPO906 alone. **P < 0.01 versus EPO906 monotreatment; *** P < 0.001 versus vehicle.

**Fig. 3** STI571 augments the tumor uptake of EPO906. The content of EPO906 was determined in tumors derived from mice treated with vehicle or STI571 and sacrificed 2 h (vehicle, n = 6; STI571, n = 5) or 24 h (vehicle, n = 6; STI571, n = 4) after injection of EPO906. ***, P < 0.001; * P < 0.05 versus vehicle.
analyzed after monotreatment with EPO906 or combination treatment with STI571 and EPO906. Treatment with STI571 did not significantly increase the uptake of EPO906 in liver, kidney, or small intestine over that seen with the monotreatment group (Fig. 5a).

To explore if combination treatment was associated with decreased tolerability, the body weight of the mice was recorded during 3 weeks of treatment (Fig. 5b). Treatment with 0.3 mg/kg EPO906 was well tolerated by the mice, causing no significant body weight loss, whereas treatment with 1.0 mg/kg EPO906 was not well tolerated, leading to an average body weight loss of 14.3% during the study (Fig. 5b). The combination of STI571 and 0.3 mg/kg EPO906 did not lead to decreased tolerability compared with 0.3 mg/kg EPO906 alone, as judged by body weight loss (Fig. 5b).

Taken together, the results in Fig. 5 demonstrate that the...
STI571-mediated potentiation of the therapeutic effects of EPO906 are not associated with increased drug uptake in other organs or reduced tolerability. Thus, STI571 cotreatment leads to an improved therapeutic index of EPO906.

The Effects of STI571 on Tumor Uptake of EPO906 and on Tumor IFP Are Transient and Correlate with Enhancement of the Antitumor Effects of EPO906. A set of experiments were performed with the double purpose of performing a more detailed characterization of the temporal requirements to obtain STI571-induced changes in tumor drug uptake and tumor IFP and to further investigate the correlations between these events and the beneficial antitumor effects of the combination treatment.

It was first analyzed to what extent the STI571-induced effects on tumor uptake of EPO906 lasted after interruption of STI571 treatment. Tumor-bearing animals were subjected to a 3-day treatment with STI571, and tumor uptake of EPO906 was subsequently determined either immediately after STI571 treatment or after a 3-day period without STI571 treatment (Fig. 6a). Results were compared with data from animals that had not received any STI571 treatment. The increased uptake observed when EPO906 was administered on the last day of a 3-day treatment with STI571 was not seen when EPO906 was given 3 days after the last dose of STI571.

The findings of a transient effect of STI571 on tumor uptake of EPO906 were followed by studies on STI571-induced reduction of tumor IFP. In this experiment, the tumor IFP was measured before treatment with STI571, after 3 days of STI571 treatment and, finally, after an additional 3 days without STI571 treatment. The dependence on STI571 of the changes in the IFP complemented those of the drug uptake study, i.e., the IFP was reduced immediately after a 3-day STI571 treatment period but had returned to baseline levels 3 days after cessation of STI571 treatment (Fig. 6b).

Subsequently, a therapeutic study with three different EPO906 and STI571 combination regimens was performed (Fig.
In all regimens, EPO906 was given once weekly at a dose of 0.3 mg/kg/week to KAT-4 tumors from mice that had received two daily doses of vehicle (n = 6) or STI571 (n = 7). The second dose was administered 1–2 h before IFP measurement. Growth of KAT-4 tumors was monitored in mice treated with vehicle (n = 6), 0.3 mg × kg⁻¹ × week⁻¹ EPO906 alone (n = 6), or together with STI571 scheduled as indicated in the top panel (combination regimen B, n = 6; combination regimen C, n = 6). * P < 0.05 combination regimen versus EPO906 alone. C, tumor IFP was measured in KAT-4 tumors from mice that had received STI571 or vehicle treatment as indicated (n = 5 in all groups). The IFP measurement was performed 1–2 h after the last dose of vehicle or STI571. * P < 0.05 group 2 versus group 1.

Two Doses of Daily Treatment with STI571 Are Insufficient to Increase Tumor Drug Uptake, Reduce Tumor IFP, or Enhance the Therapeutic Effects of EPO906. A final set of experiments were performed to extend the characterization of the STI571-induced changes of tumor drug uptake and tumor IFP and the correlations between these alterations and the beneficial effects of combination treatment. In these experiments, treatment with two or three doses of STI571 was compared.

Two doses of treatment with STI571 (first dose administered 16 h before measurement of tumor IFP or injection of EPO906), in contrast to three doses of treatment (first dose administered 48 h before measurement of tumor IFP or injection of EPO906), did not suffice to increase the tumor uptake of EPO906 (Fig. 6a) or induce a lowering of the tumor IFP (Fig. 8a). Also, the shorter treatment period with STI571 did not potentiate the antitumor effect of EPO906 on KAT-4 tumors (Fig. 8b). Furthermore, the experiments shown in Fig. 8c indi-
cata that the lack of effect on the tumor IFP of two doses of 100 mg/kg × day treatment with STI571, as compared with three doses of 100 mg/kg × day, could not be overcome by giving two doses of 150 mg/kg × day.

These experiments establish that in the KAT-4 thyroid carcinoma model system, two doses of treatment with STI571 are not sufficient to reduce tumor IFP, increase tumor uptake, or potentiate the therapeutic effect of EPO906. Furthermore, the observed effects are transient and STI571 needs to be administered continuously for at least 48 h. In addition, the data provide additional support for a causal relationship between these effects of STI571.

**DISCUSSION**

In the present investigation, it is demonstrated that the tyrosine kinase inhibitor STI571 potentiates the therapeutic effect of the novel microtubule-stabilizing agent EPO906 in a tumor model where the cancer cells per se are insensitive to STI571. The improved efficacy was matched by a 3-fold increase in the tumor levels of the chemotherapeutic compound. The enhanced tumor uptake of EPO906 observed concomitant with PDGF receptor inhibition was found to be tumor specific because STI571 treatment did not enhance the tissue levels of EPO906 in liver, kidney, or the intestinal tract. Furthermore, STI571 treatment did not decrease tolerability of EPO906, as judged by body weight. Thus, combination treatment with STI571 enhances the therapeutic index of EPO906. Moreover, characterization of the STI571-induced reduction in tumor IFP and increase in tumor uptake revealed that both effects were transient, temporally linked, and in the KAT-4 tumor model, required a minimum of three daily doses of STI571.

STI571 is a tyrosine kinase inhibitor shown to block the PDGF receptor, c-Kit, Abl, and the ARG tyrosine kinase activities with equal potency but does not act on the vascular endothelial growth factor receptors 1 or 2 (5, 14–16). Although STI571 is not completely specific for the PDGF receptor, earlier studies using an aptamer entirely specific for inhibition of PDGF receptors has produced effects similar to those reported here, making it most likely that the effects of STI571 seen in this study are indeed a consequence of blocking PDGF receptor-induced signaling (5, 6).

Increased tumor uptake of the tracer compound $^{51}$Cr-EDTA and paclitaxel, after inhibition of PDGF receptor inhibition in tumor stroma, has previously been demonstrated (5, 6). An increased tumor uptake of cytotoxic drugs has therefore been proposed as the mechanism that underlies the enhanced antitumor effects observed after combination treatment with PDGF antagonists and chemotherapy (6). The results of the detailed investigation of the kinetics of the STI571-induced increase in tumor uptake of drugs and the results from therapeutic studies assessing different temporal relationship between administration of STI571 and EPO906 strongly support the notion that the therapeutic effect of STI571 occurs as a consequence of its effect on tumor drug uptake. Immunohistochemical analyses further demonstrated that the enhanced antitumor efficacy of this regimen was matched by an increase in the level of tumor cell mitosis and apoptosis, consistent with increased tumor levels of EPO906. Indeed, the tumor levels of EPO906 after administration of 0.3 mg × kg$^{-1}$ in combination with STI571 were similar to those seen with an equiefficacious monotherapy dose of EPO906 (1 mg × kg$^{-1}$).

The mechanism whereby PDGF receptor inhibition causes an increase in tumor drug levels remains to be definitely established. However, the correlations we observe between effects of STI571 on IFP and on tumor drug uptake are in agreement with the hypothesis that the increased uptake occurs as a direct or indirect consequence of reduction in the tumor IFP. A regulatory role of PDGF β-receptor signaling in control of IFP was originally demonstrated in normal loose connective tissue, where local administration of PDGF-BB, but not PDGF-AA, was shown to normalize the negative IFP induced by anaphylactic shock (30). Subsequent studies demonstrated that this process required PDGF receptor-induced activation of phosphatidylinositol-3-kinase (31). The findings of the present study clearly argue in favor of the notion that the lowered tumor IFP is the cause of the increased uptake of the cytotoxic drug. The schedules of STI571 that caused a decrease in tumor IFP were accompanied by an increased tumor uptake of EPO906, whereas scheduling of STI571 that did not affect the tumor IFP failed to enhance tumor uptake of the cytotoxic drug. Independent observations of increased tumor drug uptake after lowering of the tumor IFP include demonstrations of increased tumor uptake of tumor-targeting antibodies and carboplatin after treatment with hyaluronidase and the bradykinin receptor agonist Cerupor, respectively (2, 8). Also, the reduction in tumor IFP induced by prostaglandin E$_1$ is paralleled by increased tumor uptake of $^{51}$Cr-EDTA (7). However, other potentially contributing effects of STI571 such as an increased blood flow rate or volume and altered permeability, cannot yet be ruled out and merits additional analyses.

It is, however, legitimate to speculate that the current preclinical finding, i.e., that STI571-mediated PDGF receptor inhibition enhances the therapeutic index of EPO906 by selectively increasing its uptake in tumor, can be clinically exploited to optimize cancer patient benefit and, in fact, might be applied to many other chemotherapeutic drugs. Therefore, the feasibility of this strategy merits additional testing in a clinical trial.

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