Hypertension and Rarefaction during Treatment with Telatinib, a Small Molecule Angiogenesis Inhibitor

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Abstract

Purpose: Hypertension is a commonly reported side effect in antiangiogenic therapy. We investigated the hypothesis that telatinib, a small molecule angiogenesis inhibitor, impairs vascular function, induces rarefaction, and causes hypertension.

Experimental Design: A side-study was done in a phase I trial of telatinib, a small molecule tyrosine kinase inhibitor of vascular endothelial growth factor receptors 2 and 3, platelet-derived growth factor receptor, and c-KIT in patients with advanced solid tumors. Measurements of blood pressure, flow-mediated dilation, nitroglycerin-mediated dilation, aortic pulse wave velocity, skin blood flux with laser Doppler flow, and capillary density with sidestream dark field imaging were done at baseline and after 5 weeks of treatment. Blood pressure and proteinuria were measured weekly.

Results: Mean systolic and diastolic blood pressure values increased significantly at +6.6 mm Hg (P = 0.009) and +4.7 mm Hg (P = 0.016), respectively. Mean flow-mediated dilation and mean nitroglycerin-mediated dilation values significantly decreased by -2.1% (P = 0.003) and -5.1% (P = 0.001), respectively. After 5 weeks of treatment, mean pulse wave velocity significantly increased by 1.2 m/s (P = 0.001). A statistically significant reduction of mean skin blood flux of 532.8% arbitrary units was seen (P = 0.015). Capillary density statistically significantly decreased from 20.8 to 16.7 capillary loops (P = 0.015). Proteinuria developed or increased in six patients during telatinib treatment.

Conclusion: The increase in blood pressure observed in the treatment with telatinib, an angiogenesis inhibitor, may be caused by functional or structural rarefaction.

Dysregulated signaling through the vascular endothelial growth factor (VEGF)/VEGF receptor-2 (VEGFR-2) pathway mediates neoangiogenesis and thereby promotes tumor development and metastasis (1, 2). Overexpression of VEGF is common in solid tumors and has been associated with poor prognosis (3). Furthermore, the overexpression or increased activation of VEGFR-2 has been associated with a poor prognosis in solid tumors (4, 5). In preclinical models, inhibition of the tyrosine kinase activity of the VEGFR-2 blocks angiogenesis and inhibits the growth of tumors (6).

Hypertension is a commonly reported side effect in trials with inhibitors of VEGF/VEGFR-2 signaling, like bevacizumab and sunitinib (7–12). The mechanisms leading to this increase in blood pressure during antiangiogenic therapy have not been elucidated. Proposed mechanisms include reduced formation of nitric oxide (NO) by endothelial cells, a reduced responsiveness of vascular smooth muscle cells to NO, an increased production of or reaction to vasoconstricting stimuli, a reduced compliance and distensibility of the vascular wall, and microvascular rarefaction (13–15). Because microvessels (arterioles and capillaries) are a major contributor to total peripheral vascular resistance, functional rarefaction (a decrease in perfused microvessels) or anatomic rarefaction (a reduction in capillary density) may play an important role in the development of hypertension.

We hypothesized that systemic inhibition of VEGF impairs vascular function and causes rarefaction, which then leads to the development of hypertension in patients treated with antiangiogenic agents.

Materials and Methods

This study was conducted on a subset of patients enrolled into an open-label, nonrandomized, two-center, phase I dose-escalating study of oral telatinib (Bay 57-9352; ref. 16). The purpose of this study was to search for possible mechanisms that cause hypertension in patients treated with antiangiogenic therapy and to confirm our hypothesis that

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systemic inhibition of VEGF inhibits vascular function and causes rarefaction.

Patients

Patients with advanced solid tumors with no standard treatment available were eligible for study participation. Inclusion criteria were age of 18 y or older; WHO performance status of 0 to 2; life expectancy of at least 12 wk; and adequate bone marrow, liver, and renal function. Exclusion criteria were history of cardiac disease; history of HIV, hepatitis B, or hepatitis C infection; active clinically serious infection; serious nonhealing wound, ulcer, or bone fracture; symptomatic metastatic brain or meningeal tumors; pregnancy or breast feeding; treatment with any anticancer agent or investigational drug 4 wk before the first dose; antiangiogenic therapies/VEGFR-2 inhibitors before enrollment.

The side-study was conducted on patients that were treated in the Leiden University Medical Center. The study protocol was approved by the Medical Ethical Committee of the Leiden University Medical Center. All patients gave written informed consent.

Intervention

Telatinib (Bay 57-9352) is an orally active, small molecule inhibitor of the VEGFR-2 (IC_{50} in biochemical assay, 6 nmol/L), VEGFR-3 (IC_{50}, 4 nmol/L) tyrosine kinases, and the growth factors receptors platelet-derived growth factor receptor-α (IC_{50}, 15 nmol/L) and c-Kit (IC_{50}, 1 nmol/L). Telatinib was continuously given once daily or twice daily in an oral formulation as solution or tablet. Patients were divided into cohorts with escalating doses. Therapy continued until progressive disease, unacceptable toxicity, death, consent withdrawal, or withdrawal from study at the discretion of the investigator. Telatinib was provided by Bayer Pharmaceuticals Corporation.

We assessed blood pressure, vascular function, and structure variables at baseline, and after 5 wk of treatment, in addition to regular evaluation of variables for safety, pharmacokinetics, and efficacy.

Hemodynamic, vascular function, and vascular structure variables and proteinuria

Blood pressure, flow-mediated dilation (FMD), nitroglycerin-mediated dilation (NMD), aortic pulse wave velocity (PWV), skin blood flux with laser doppler flow, and capillary density with sidestream dark field (SDF) imaging were assessed at baseline and after 5 wk of treatment with telatinib. All measurements were done by the same experienced investigator, in the morning, in a quiet, temperature-controlled room.

Peripheral blood pressure measurements were also done at every weekly visit to the outpatient clinic.

Peripheral blood pressure. Peripheral blood pressure measurements at baseline and at the 5-wk visit were done after 15 min rest, measuring thrice in a supine position with 5-min intervals, using an automatic device (Datex-Ohmeda S/5 Light Monitor, Datex-Ohmeda, Inc.) with the cuff placed at the brachial artery. For statistical analysis, we used the mean of three consecutive measurements.

Peripheral blood pressure measurements at the weekly visit to the outpatient clinic were done by the treating physician, using an aneroid sphygmomanometer (Maxi-Stabil 3, Speidel & Keller, Welch Allyn) with the auscultatory method.

Central blood pressure. Application tonometry of the brachial and external carotid artery (SphygmoCor SCOR-PVx device, AtCor) was done. The mean of the three peripheral blood pressure measurements was used to calculate central aortic pressure (17).

Aortic pulse wave velocity. Measurements were done at the right carotid and femoral arteries using standard blood pressure transducers (SphygmoCor SCOR-PVx device, AtCor) with simultaneous electrographic gating. This enabled the base of the pressure wave to be recorded and the time delay between the carotid and femoral waves to be calculated. The distance between the two sites was measured. PWV was defined as the distance traveled by the pressure waves divided by the time delay.

Flow mediated dilation. The FMD measurements were done in a quiet, temperature-controlled room. Postischemic vasodilator responses in the brachial artery were measured using a Wall Track System (WTS 2, Pie Medical). This system consists of a standard 7.5-MHz linear array ultrasound transducer connected to a PC equipped with a data acquisition board and software. Subjects were investigated in a supine position, and three ECG leads were attached. Ischemia was induced in the forearm by inflation of a blood pressure cuff just below the elbow of the right arm for 5 min. After deflation of the cuff, changes in brachial artery wall diameter were measured every 20 s for 4 min. WTS measurements were stored and analyzed off line using WTS software. FMD was expressed as percentage change in brachial artery diameter after ischemia.

NMD. NMD was assessed in the same way as FMD, with the exception that 0.4 mg of nitroglycerin were given sublingually, instead of cuff inflation and deflation, before measurements were started.

Laser Doppler flowmetry. Forearm skin blood flux was measured using laser Doppler flowmetry (Periflux PF4001, Perimed; wavelength, 782 nm) before and during forearm postischemic hyperemia (18). Flows were recorded by the Perisoft program, with the time constant set at 3 s downstream from a broadband filter (12 MHz). Results were reported as arbitrary flow units (10 mV). The percentage of change in arbitrary units from baseline (before ischemia) to maximal flow in the postischemic hyperemic phase was reported.

Capillary density measurements with SDF imaging. Patients were situated in a supine position with the investigator at the head side of the bed. An SDF hand-held device (MicroScan Video Microscope System, MicroVision Medical) was introduced into the open mouth and gently pushed to the mucosal surface of the inner lip. SDF imaging consists of a light guide surrounded by light-emitting diodes that emit green light (540 ± 50 nm) which penetrates the tissue and directly illuminates the tissue microcirculation. The SDF technique and the technique of its precessor orthogonal spectral polarization imaging are described in detail in previous publications (19, 20).

Images of the mucosal microcirculation were projected on a computer screen. The final on-screen magnification of the images obtained with the SDF imaging device was 325 times original. When images of satisfying quality were seen, video images of at least 30 s were obtained. Images were obtained from four different lip quadrants (mucosal readings of the left and right upper inner lip quadrant and the left and right lower inner lip quadrant) using the SDF probe. From every quadrant, at least three 30-s video images were obtained. Video images were stored on digital videotape in .avi format.

Off line, at least five still frames of each quadrant were captured from these video images. The number of capillary loops per frame was counted. Capillary density for each frame was expressed as the mean number of capillary loops per mm^2. The mean capillary density per lip quadrant and total lip was calculated.

All measurements were done by one technician, not blinded to the time point in treatment of the patients. Off-line analysis (counting of the number of capillary loops) was done by two observers, who were blinded to the time point in treatment of the patients.

Whereas the technique has not been used very frequently in the measurement of microcirculation of the mucosal surface of the inner lip, additional quality measurements were done. In 10 healthy volunteers, no difference in capillary density was observed between the different lip quadrants. The reproducibility of the SDF technique to determine capillary density was moderate to high, showing a coefficient of variation of 4.6%.

Proteinuria. Urinalysis, measured by dipstick, was done weekly in all patients to monitor proteinuria. Proteinuria was recorded according to the National Cancer Institute Common Toxicity Criteria version 3.0. Grade 1 is defined as 1+ by dipstick, grade 2 as 2+ or 3+ by dipstick, grade 3 as 4+ by dipstick, and grade 4 as nephrotic syndrome. We report the development of proteinuria (grade 0 before treatment increasing to grade >0 during treatment) and the worsening of proteinuria (increase of proteinuria by $\geq$1 grade compared with baseline).

Pharmacokinetic analysis

Serial blood samples were collected for pharmacokinetic analysis on days 1 and 14 of cycle 1. Telatinib plasma concentrations were analyzed by a noncompartmental method using the KINCALC software package, Bayer AG, version 2.33 or higher. Peak plasma level (C_{max}), area under the concentration-time curve [$AUC_{(0\text{-}tn)}$], were calculated.

Statistical analysis.

Continuous variables are presented as mean values ± SD and categorical variables as frequencies (percentages), unless otherwise stated. Comparisons between variables at baseline and after 5 wk were done with paired *t* tests and were two-sided, with a level of significance of $\alpha = 0.05$. For skin blood flux and capillary density, the Wilcoxon signed-rank test was used. The relationship between blood pressure, vascular function and structure variables, and telatinib daily dose and telatinib pharmacokinetic variables [C_{max} and $AUC_{(0\text{-}tn)}$] was investigated by correlation analysis. Correlation analysis was done using Pearson's and Spearman's correlation coefficients where appropriate. Correlations with proteinuria were done using an armitage test for trend. For correlation purposes proteinuria was reported as presence of new proteinuria or increase in existing proteinuria (yes or no). All analyses were done using SPSS version 12.01 (SPSS).

Results

Eighteen of 33 patients treated in our hospital were included in this side study. Reasons for exclusion were vaso-active hormone producing adrenal carcinoma ($n = 3$), absence of measurements for logistics reasons between June and December 2005 ($n = 7$), absence of measurements at 5 weeks due to early drop out for early progressive disease ($n = 2$), anatomic anomaly of the arm ($n = 1$), absence of appropriate drug compliance (supported by pharmacokinetic data; $n = 1$), and failure to upheld appointment baseline visit ($n = 1$). NMD measurements were not done in two patients; both had a preexisting headache and refused sublingual nitroglycerin administration.

Baseline demographics and patient characteristics of the 18 patients included in this study are listed in Table 1. Patients received the following starting doses of Bay 57-9352: patient 1, 20 mg solution once daily; patients 2 to 3, 75 mg (25 mg tablets) once daily; patients 4 to 5, 150 mg (150 mg tablets) twice daily; patients 6 to 9, 300 mg (150 mg tablets) twice daily; patient 10, 600 mg (150 mg tablets) twice daily; and patients 11 to 18, 900 mg (150 mg tablets) twice daily.

Blood pressure results. Both peripheral systolic blood pressure and peripheral diastolic blood pressure increased in 14 of 18 patients (78%) after 5 weeks treatment with telatinib compared with baseline values. The mean peripheral systolic blood pressure significantly increased from 132.2 to 138.8 mm Hg ($P = 0.009$), and the mean peripheral diastolic blood pressure values increased from 83.1 to 87.8 mm Hg ($P = 0.016$; Table 2; Fig. 1A). The increase in central systolic blood pressure (4.3 mm Hg) was not statistically significant ($P = 0.106$). Both peripheral and central pulse pressure showed no change after 5 weeks of treatment.

Mean peripheral blood pressures measured at the weekly visits showed a similar increase in both systolic and diastolic blood pressure (Fig. 1B). Blood pressure results for the individual patients are reported in Table 2B. Results for the first 84 days on treatment are reported. The number of patients on telatinib treatment after 84 days was too small for reliable results to be reported ($n = 7$). None of the seven patients remaining on study medication after 84 days developed a new increase in blood pressure. In all patients, the blood pressure values returned to baseline within 4 weeks after the discontinuation of the telatinib.

Table 1. Baseline demographics and patient characteristics

Baseline characteristics	
N	18
Male gender	9 (50)
Age, y (range)	55 (22-76)
Additional cardiovascular risk factors	
Body mass index, kg/m^2 (range)	24.7 (20.5-29.7)
Nicotine abuse, in past or present	5 (28)
History of cardiovascular disease	0 (0)
History of hypertension	1 (6)
Renal impairment (creatinine > ULN)	5 (28)
WHO performance scale	
0	4 (22)
1	14 (78)
Prior treatment	
Surgery	13 (72)
Chemotherapy	17 (94)
Radiotherapy	8 (44)
Blood pressure lowering drugs at entry	2 (11)
Tumor type	
Anal carcinoma	1 (6)
Carcinoid tumor	1 (6)
Cholangiocarcinoma	1 (6)
Colorectal carcinoma	3 (17)
Esophageal carcinoma	1 (6)
Ovarian carcinoma	3 (17)
Prostatic carcinoma	1 (6)
Renal cell carcinoma	1 (6)
Soft tissue sarcoma	5 (28)
Urothelial cell carcinoma	1 (6)

NOTE: Data are presented as *n* (%) unless otherwise specified.
Abbreviation: ULN, upper limit of normal.

One patient received antihypertensive medication before start of treatment (thiazide diuretic). Four additional patients were started on antihypertensive treatment: one patient receiving 600 mg telatinib daily and three patients receiving 1800 mg daily. Antihypertensive medication consisted of a thiazide diuretic in one patient, a calcium antagonist in one patient, and an ACE inhibitor in two patients.

Vascular function and vascular structure assessments. FMD decreased from baseline in 15 of 18 patients (83%) after 5 weeks treatment with telatinib. At 5 weeks, the mean decrease in FMD, compared with baseline, was statistically significant, from 6.0% to 3.9% ($P = 0.003$; Table 2). After 5 weeks of treatment, NMD decreased in 94% of patients. The mean change in NMD from 17.0% at baseline to 11.9% after 5 weeks was statistically significant ($P = 0.001$; Table 2). An increase in PWV was seen in 17 of 18 patients (94%). Mean PWV significantly increased from 8.5 m/s at baseline to 9.7 m/s after 5 weeks treatment ($P = 0.001$; Table 2). Mean forearm skin blood flux decreased significantly (-532.8 %AU, $P = 0.015$; Table 2). SDF imaging was done in seven patients. In all of the patients, the number of capillary loops markedly decreased after 5 weeks of treatment (Figs. 1 and 2; Table 2). Capillary density, the mean number of capillary loops per image, decreased from 20.8 at baseline to 16.7 after 5 weeks treatment with telatinib ($P = 0.015$).

Table 2.

A. Hemodynamic and vascular function/structure variables at baseline and after 5 wk of treatment with telatinib

	Baseline values	After 5 wk treatment	Change ± SD	95% Confidence interval	*P*
pSBP (mm Hg)	132.2	138.8	+6.6 ± 9.5	(1.9 to 11.3)	0.009*
pDBP (mm Hg)	83.1	87.8	+4.7 ± 7.4	(1.0 to 8.4)	0.016*
cSBP (mm Hg)	129.9	134.2	+4.3 ± 10.9	(-1.0 to 9.8)	0.106
cDBP (mm Hg)	82.9	87.5	+4.6 ± 7.8	(0.7 to 8.4)	0.024*
MAP (mm Hg)	102.5	107.6	+5.1 ± 7.2	(1.5 to 8.7)	0.008*
pPP (mm Hg)	54.3	57.1	+2.8 ± 12.9	(-3.6 to 9.2)	0.369
cPP (mm Hg)	47.0	46.8	-0.2 ± 10.2	(-5.2 to 4.9)	0.946
FMD (%)	6.0	3.9	-2.1 ± 2.6	(0.8 to 3.5)	0.003*
NMD (%)	17.0	11.9	-5.1 ± 4.1	(2.9 to 7.3)	0.001*
PWV (m/s)	8.5	9.7	+1.2 ± 0.8	(0.8 to 1.7)	0.001*
Skin blood flow (%AU)	1091.5	558.7	-532.8 ± 362.0	(-912.7 to -152.9)	0.015*
Capillary density (*n*)	20.8	16.7	-4.1 ± 3.3	(-7.2 to -1.1)	0.015*

B. Individual blood pressure data before treatment, during treatment, and after discontinuation of telatinib treatment

Patient	Systolic blood pressure, d														
	0	7	14	21	28	35	42	49	56	63	70	77	84	+7	+28
1	110	105	110	115	115	110	130		120	115		120	115		110
2	115	115	120	120	125	120	115							130	120
3	130	160†	135	140	150	128	150		180†	145		145		135	
4	130	125	120	138	120	125	125								
5	115	120	125	120	120	125	130								110
6	145	150	145	155†	140		140		135	130		160†	150		120
7	110	100	120	125	110		110			120		125	105		110
8	130	140	150	150	*160*	160	130			130		125	140		110
9	105	130	130	140	125	130	110								
10	130	120	126	140	135	120	140			130		135	130	140	100
11	120	120	130	130	135		120								
12	140	130	135	130	125	170†	130			*160*		149	163	150	110
13	120	125	150	140		156†	140		150	130		130			130
14	125		143	130	130	112									120
15	120	125	110	120	120	135	120								120
16	135	130	*150*	145	130	110								118	
17	130	125	130	135	120	128	125								
18	125	140	145	*150*	125	130	125		140	135		105	130	Ongoing	

	Diastolic blood pressure, d														
	0	7	14	21	28	35	42	49	56	63	70	77	84	+7	+28
1	60	60	55	70	55	70	65		60	60		70	55		70
2	70	80	80	80	80	80	80							80	80
3	75	80	75	80	70	72	70		80	80		70		75	
4	85	90	90	85	65	60	70								
5	75	80	75	85	80	75	80								70
6	85	85	85	85	80		90		85	85		90	90		80
7	70	70	75	85	85		80			80		80	70		70
8	75	70	80	90	90	89	85			75		80	80		60
9	70	90	85	90	80	80	70								
10	75	80	81	85	80	85	70			85		80	75	65	70
11	75	75	80	85	85		80								
12	80	80	85	80	80	102†	90			*100*		100	98	85	75
13	70	80	89	85		97	90		90	80		80			75
14	80		79	85	80	78									75
15	75	80	70	70	80	80	80								80
16	85	90	*110*	90	75	70								77	
17	85	80	85	90	80	74	80								
18	80	85	85	90	85	85	80		90	90		70	80	ongoing	

NOTE: Data in italics indicate antihypertensive medication started.
Abbreviations: pSBP, peripheral systolic blood pressure; pDBP, peripheral diastolic blood pressure; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; MAP, mean arterial pressure; pPP, peripheral pulse pressure; cPP, carotid pulse pressure; %AU, percentage of change from baseline in arbitrary units; *n*, number.
*$P < 0.05$.
† No antihypertensive treatment started, regardless of protocol.

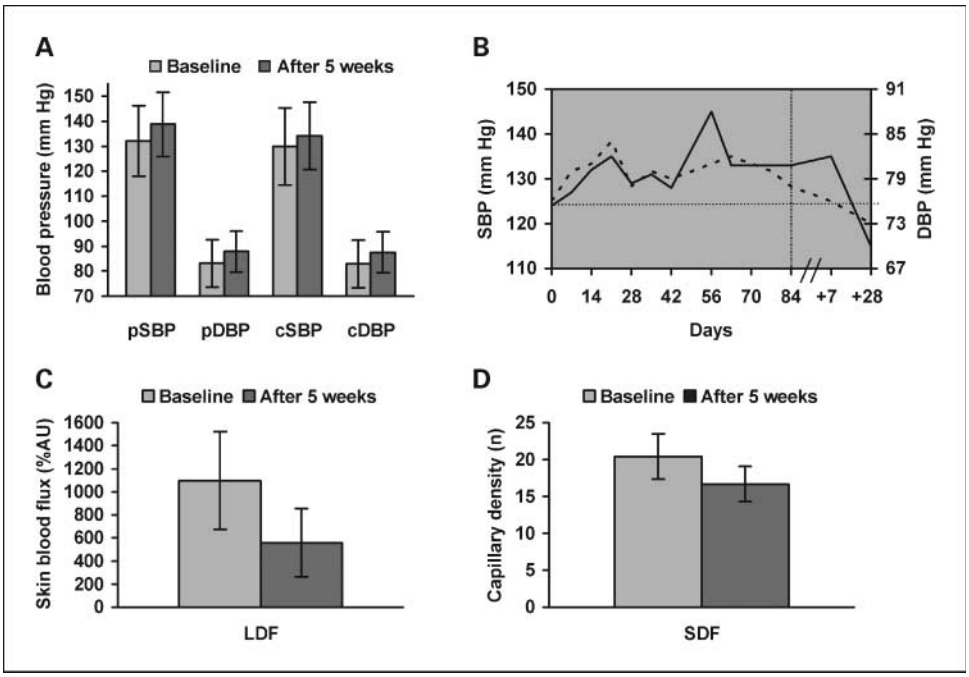

Fig. 1. Blood pressure (*A*), skin blood flux (*C*), and capillary density (*D*) results at baseline and after 5 wk of treatment with telatinib. *B,* mean systolic blood pressure (*continuous line*) and mean diastolic blood pressure (*dashed line*) before treatment, weekly during treatment, and after discontinuation of telatinib treatment. A horizontal dashed line was added at baseline systolic blood pressure and baseline diastolic blood pressure for facilitation of reading. Left from the vertical line blood pressures measured in the first 84 d of treatment. Right from the vertical line blood pressures measured 7 and 28 d after discontinuation of treatment. pSBP, peripheral systolic blood pressure; pDBP, peripheral diastolic blood pressure; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; LDF, laser doppler flow; %AU, percentage of change from baseline in arbitrary units; *n*, number.

Proteinuria. In four patients, proteinuria was reported at baseline, grade 1 proteinuria in one patient, and grade 2 proteinuria in three patients. Proteinuria increased in one of those patients from grade 1 to grade 2. Five patients developed new onset proteinuria during telatinib treatment: grade 1 in three patients and grade 2 in two patients. Five of these six patients with new onset or increasing proteinuria were receiving the highest dose of telatinib at 1,800 mg daily. After discontinuation of treatment in three of six patients, the proteinuria returned to normal. For the other three patients, no data for proteinuria after discontinuation of telatinib were available. In two of the six patients with new or increasing proteinuria, an increase in blood pressure above 150 mm Hg systolic or above 100 mm Hg diastolic was reported. These two patients were treated with an ACE inhibitor, resulting in a disappearance of the proteinuria. The other four patients were not treated for the proteinuria.

Pharmacokinetic analysis and correlations. Telatinib pharmacokinetic variables [C_{max} and $AUC_{(0\text{-}tn)}$] are shown in Table 3. There was no correlation between either blood pressures or vascular function/structure variables and daily dose of telatinib or telatinib pharmacokinetic variables [C_{max} and $AUC_{(0\text{-}tn)}$]. No correlation between development or increase of proteinuria and blood pressure measurements or any of the other variables was seen. However, there was a positive correlation between daily dose of telatinib and proteinuria (linear-by-linear association, 5.0; $P = 0.025$). All patients with SDF measurements done received 1,800 mg of telatinib a day. No correlation between SDF results and daily dose could therefore be calculated.

Discussion

We studied the effects of telatinib, a tyrosine kinase inhibitor and potent inhibitor of angiogenesis, on the vasculature to determine a mechanism by which small molecule angiogenesis inhibitors cause an increase in blood pressure. The rarefaction (reduction in capillary density) and change in microvascular characteristics observed in this study provide a plausible mechanism for the increase in systolic and diastolic blood pressure.

Telatinib caused a significant decrease in endothelium-dependent and endothelium-independent vasodilation. VEGF inhibition by itself decreases NO synthesis, which promotes vasoconstriction, increases peripheral resistance, and therefore can induce an increase in blood pressure (21–24). It remains unclear whether the key problem is impaired NO synthesis, the change in capillary structure leading to impaired NO vascular smooth muscle cell responsiveness, or a combination of both.

Aortic pulse wave velocity is a variable for vascular stiffness, which is known to increase with age, and is an independent predictor of cardiovascular risk and all-cause mortality in renal disease, hypertensive patients, and patients with diabetes mellitus (25–27). We observed a significant increase in PWV, which correlated with the increase in mean arterial pressure. Although blood pressure is a known independent determinant of pulse wave velocity, it cannot be excluded that inhibition of angiogenesis has a direct effect on stiffness of the arterial tree (28).

In a subgroup of patients, we did SDF imaging to visualize the microvessels in the buccal mucosa. All patients showed a reduction in the number of mucosal capillaries (rarefaction) during antiangiogenic treatment. Vessels smaller than 150 μm in diameter are the most important segment of the vascular bed to regulate blood flow and blood pressure (29, 30). A reduction in the number of (functional) arterioles and capillaries leads to increased peripheral vascular resistance and blood pressure.

Rarefaction is a consistent finding in patients with hypertension (30–32), and it is also reported in normotensive young adults with a genetic predisposition to high blood pressure (33). Blocking the growth of capillaries by VEGFR inhibitors

and other angiogenesis inhibitors might lead to the same results even in subjects that are not predisposed to the development of hypertension. Whether the observed rarefaction is structural (disappearance of capillaries) or functional (i.e., nonperfused existing capillaries) is unclear, as visualization of microvessels based upon the SDF technique depends on perfusion of these vessels. Although the rapid normalization of blood pressure within weeks and reversal in proteinuria in some patients after discontinuation of telatinib may indicate improvement in functional rarefaction, this is more likely in functional then structural rarefaction. It remains uncertain whether the changes in microvessel architecture are reversible upon discontinuation of the treatment. While capillary density measurements were done in only seven patients, one should be careful with the interpretation of these results. These results have to be confirmed in a larger patient sample.

The exact mechanism by which telatinib leads to rarefaction and hypertension is unclear. Telatinib is a small molecule tyrosine kinase inhibitor, blocking the ATP-binding site of the VEGFR-2, VEGFR-3, platelet-derived growth factor receptor-α, and c-Kit receptors. Platelet-derived growth factor and c-Kit receptor activation result in activation of pathways that, for a large part, are also activated by VEGFR-2. However, hypertension is rarely seen in the treatment with platelet-derived growth factor and c-Kit inhibitors, such as imatinib and nilotinib (34, 35). In contrast, selective inhibitors of VEGF/VEGFR-2 signaling, such as sunitinib or bevacizumab, frequently cause hypertension (7–10). The increase in blood pressure is therefore most likely caused by the inhibition of the VEGFR signaling. However, we cannot rule out that c-KIT or platelet-derived growth factor inhibition has a role in mediating the blood pressure changes or changes in any of the other measured variables. A recently published preclinical observation suggests that VEGF signaling is required for vascular homeostasis (36). Our findings could be the clinical proof of that concept.

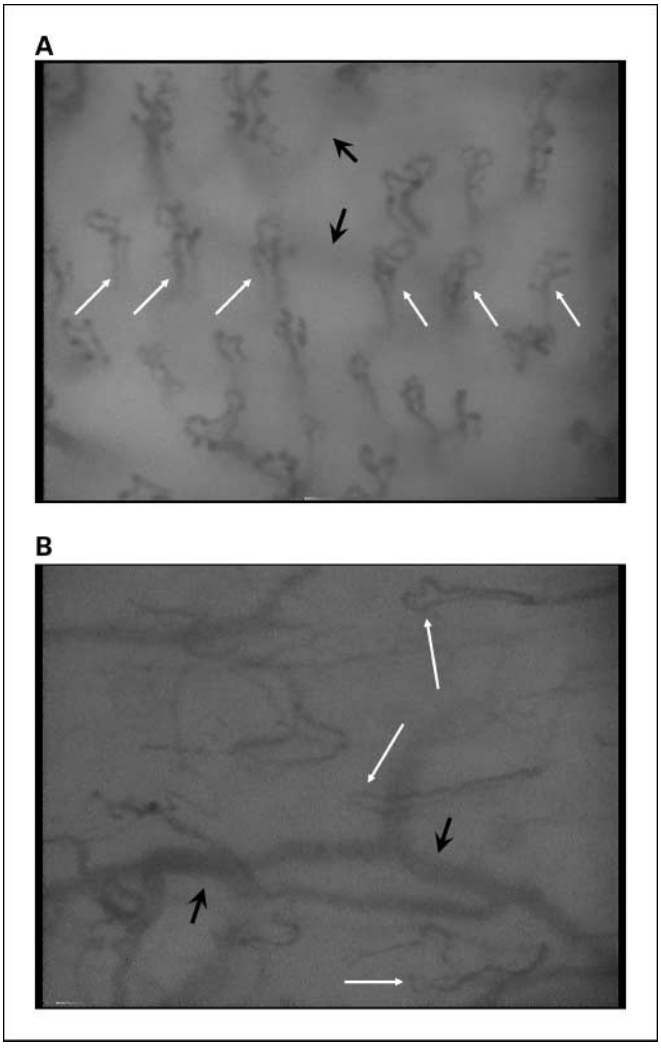

Fig. 2. SDF images demonstrating visible capillary loops of a representative patient. *A,* at baseline. *B,* after 5 wk of telatinib treatment. Black arrows, larger venules; white arrows, individual superficial capillary loops.

Our study has several limitations. First, the study was set up as a side-study of a phase I dose-finding study. Therefore, different dosages of telatinib were used by our patients. However, there was no correlation between changes on blood pressure, vascular structure/function variables, capillary density, and daily dose of telatinib or telatinib exposure. Even in the patients with lower doses of telatinib, significant changes in all measured variables were seen. Second, due to the small number of patients it was not possible to reliably quantitate capillary characteristics, such as length, diameter size, and tortuosity. Third, no control group was measured and distinction between treatment and placebo effects is therefore not clear. Fourth, no vascular measurements were done after discontinuation of treatment. Whereas all patients had advanced tumors with a low life expectancy, we chose not to burden these patients with additional measurements after cessation of the study drug. Finally, the temporal relationship between rarefaction and hypertension is unclear. Therefore, future studies, in larger patient samples, with measurements before, during, and after treatment are necessary.

In the most extensively studied VEGF inhibitor bevacizumab, the increase in blood pressure is dose dependent (13). We did not observe this in our study. This could have been due to the small study size. In addition, the start of antihypertensive medication may have masked a correlation between blood pressure and daily dose of telatinib. However, the development or increase of proteinuria was dose dependent. Another explanation for the sole dose dependency for proteinuria is that telatinib may have an effect on glomerular endothelial cells, which is independent of blood pressure and independently caused by the VEGF blockade (37–40).

In conclusion, we report that 5 weeks of treatment with a small molecule tyrosine kinase inhibitor, blocking VEGFR-2 and VEGFR-3, results in a significant increase in both systolic and diastolic blood pressure. The reduction in capillary density and microvascular flow, associated with a reduced vasodilatory capacity, may suggest that rarefaction is a mechanism that underlies the increase in blood pressure induced by telatinib and possibly other antiangiogenic agents. Further research in larger patient samples is needed to confirm this hypothesis.

Disclosure of Potential Conflicts of Interest

O. Christensen, P. Rajagopalan are or have been employed by Bayer Pharmaceuticals Corp.

Table 3. Telatinib daily dose and pharmacokinetic variables [C_{max} and $AUC_{(0\text{-}tn)}$]

Patient	Daily dose (mg)	Cycle 1, day 1		Cycle 1, day 14	
		C_{max}, mg/L	$AUC_{(0\text{-}tn)}$, mg h/L	C_{max}, mg/L	$AUC_{(0\text{-}tn)}$, mg h/L
1	20	0.2061	0.5284	0.1658	0.9628
2	75	0.2927	1.7464	0.1888	1.8779
3	75	0.3734	1.4246	0.4526	1.6941
4	300	0.0675	0.4141	0.1822	1.7680
5	300	0.0935	0.7527	0.1355	1.0164
6	600	1.2678	3.4588	1.1915	5.9823
7	600	0.1250	0.4504	0.6493	3.3407
8	600	0.1888	1.1649	0.2897	2.3776
9	600	1.2809	8.7848	1.4486	10.3795
10	1200	2.1691	16.7094	1.6995	16.4708
11	1800	0.4298	2.4610	1.7637	5.2292
12	1800	1.0484	7.0520	1.1216	7.3369
13	1800	0.2856	3.0094	0.2229	1.9701
14	1800	0.0552	0.8416	0.0969	0.8832
15	1800	0.2918	2.7700	0.8145	6.7397
16	1800	1.2599	3.4356	0.5728	3.1803
17	1800	1.6730	9.7474	2.6011	12.2049
18	1800	0.4515	2.9511	0.7626	6.2329

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