Hypertension Induced by the Tyrosine Kinase Inhibitor Sunitinib Is Associated With Increased Circulating Endothelin-1 Levels

Mariëtte H.W. Kappers, Joep H.M. van Esch, Wim Sluiter, Stefan Sleijfer, A.H. Jan Danser, Anton H. van den Meiracker

Abstract—Angiogenesis inhibition with sunitinib, a multitarget tyrosine kinase inhibitor of the vascular endothelial growth factor receptor, is associated with hypertension and cardiac toxicity, of which the underlying pathophysiological mechanism is unknown. We investigated the effects of sunitinib on blood pressure (BP), its circadian rhythm, and potential mechanisms involved, including the endothelin-1 system, in 15 patients with metastatic renal cell carcinoma or gastrointestinal stromal tumors. In addition, we investigated in rats the effect of sunitinib on BP, serum endothelin-1 levels, coronary microvascular function, cardiac structure, and cardiac mitochondrial function. In patients, BP increased by \(\approx 15\) mm Hg, whereas heart rate decreased after 4 weeks of treatment. Furthermore, the nocturnal dipping of BP diminished. Plasma endothelin-1 concentration increased 2-fold \(P<0.05\) and plasma renin decreased \(P<0.05\), whereas plasma catecholamines and renal function remained unchanged. In rats, 8 days of sunitinib administration induced an \(\approx 30\)-mm Hg rise in BP, an attenuation of the circadian BP rhythm, and a 3-fold rise in serum endothelin-1 and creatinine, of which all but the rise in creatinine reversed after sunitinib withdrawal. Coronary microvascular function studies after 8 days of sunitinib administration showed decreased responses to bradykinin, angiotensin II, and sodium nitroprusside, all normalizing after sunitinib withdrawal. Cardiac structure and cardiac mitochondrial function did not change. In conclusion, sunitinib induces a reversible rise in BP in patients and in rats associated with activation of the endothelin-1 system, suppression of the renin-angiotensin system, and generalized microvascular dysfunction. (Hypertension. 2010;56:675-681.)

Key Words: endothelin ■ endothelial growth factors ■ hypertension ■ experimental ■ angiogenesis ■ NO

Angiogenesis, the formation of new capillaries from an existing vasculature, is critical to tumor growth, as well as metastasis. This process is regulated by numerous growth factors and their receptors, among which vascular endothelial growth factor (VEGF) and its corresponding receptors play key roles. Angiogenesis inhibition as a therapeutic strategy against malignancies was first proposed by Folkman in 1971.1 Meanwhile, a variety of drugs, targeting VEGF or its receptors, have been approved for the treatment of several tumor types. Unfortunately, angiogenesis inhibition is associated with adverse effects, in particular, hypertension, which has been reported in \(\approx 60\)% of patients treated with sunitinib, an orally active multitarget VEGF receptor tyrosine kinase inhibitor (RTKI) and one of the most commonly used angiogenesis inhibitors.2 Decreased NO availability might underlie this phenomenon.3,4

VEGF inhibition with sunitinib is also associated with cardiac toxicity, as evidenced by a decrease in left ventricular ejection fraction in \(\approx 28\)% of patients.5 Given the sunitinib-induced changes in cardiac mitochondrial structure, this could relate to impaired ATP generation secondary to mitochondrial dysfunction.5 However, sunitinib, at clinically relevant concentrations, did not impair mitochondrial function in a rat myoblast cell line.6

The occurrence of the mentioned adverse effects can be a reason to lower the sunitinib dose and sometimes even to stop therapy, thereby compromising its potential efficacy. For optimal management, it is important to improve insight into the mechanisms underlying the sunitinib-induced toxicities. Our research, involving both patients and rats, was aimed to elucidate the pathophysiological mechanism(s) involved in the development of hypertension during sunitinib treatment. Simultaneously, we addressed the question as to whether and to what extent sunitinib affects cardiac structure and cardiac mitochondrial function.

Methods

Clinical Study

Between January 2008 and January 2009, patients with either metastatic renal cell carcinoma or imatinib-resistant gastrointestinal
stromal tumor, who were eligible for treatment with sunitinib, were invited to participate in our study. Patients were followed for 10 weeks during treatment with sunitinib, which was taken according to a 4 weeks "on," 2 weeks "off" regimen with a starting dose of 50 mg/d. According to the judgment of the patient's physician this dose could be adjusted. If blood pressure (BP) increased to stage II hypertension or above, antihypertensive treatment was initiated with a calcium channel blocker, an angiotensin (Ang)-converting enzyme inhibitor or an Ang II type 1 receptor blocker and a diuretic as a first, second, and third choice. Nocturnal dipper of BP was defined as a >10% decrease in systolic, diastolic, or mean arterial pressure during sleep. Patients visited the outpatient clinic every 2 weeks. At baseline and at the end of the first and second treatment cycles, 24-hour ambulatory BP measurements were performed (Suntech Oscar 2 ABP monitor and AccuwinPro soft). During the visits, sitting BP was measured during 30 minutes at 5-minute intervals using an automated device (Dynamap, Critikon Inc, model 8101) in a private room. Blood samples for laboratory measurements were obtained from an intravenous line at baseline and at the end of the first and second treatment cycles, after a 30-minute rest period. Twenty-four–hour urine samples for measurement of protein, creatinine, and sodium were collected at baseline and at weeks 4, 6, and 10. The study was approved by the institutional review board and ethical committee of the Erasmus MC in Rotterdam. Written informed consent was obtained from each patient.

**Rat Study**

Male Wistar-Kyoto rats (280 to 300 g), obtained from Charles River, were housed in individual cages and maintained on a 12-hour light/dark cycle, having access to standard laboratory rat chow and water ad libitum. Intra-aortic BP recordings were performed by radiotelemetry, for which a transmitter (TA11PA-C40, Datascience Inc) was implanted into the abdominal cavity. Telemetric data were recorded and digitized using the Dataquest Acquisition and Analysis system (DQ ART 3.1 Silver, Datascience Inc). Each animal was sampled for 10 seconds at 10-minute intervals. All of the recordings were averaged for the day and night periods. Baseline values of mean arterial pressure (MAP) and heart rate (HR) were averages of 3-day recordings before treatment was started. One week after recovery the rats were acclimatized to drug administration by oral gavage, by administering water for 8 days. The content of sunitinib-l-malate capsules, obtained from patients who discontinued treatment, was dissolved in HCl (0.1 mol/L), containing 0.5% polysorbate and 10% polyethylene glycol, after which NaOH (0.1 mol/L) was added to adjust pH to 3.5–3.7. Two separate experiments were performed. In the first experiment, rats were randomly administered sunitinib (26.7 mg/kg per day of sunitinib-l-malate; n=10) or vehicle (see above; n=10). Every oral gavage (0.5 mL) for 8 days and were euthanized with 60 mg/kg of pentobarbital IP at the end of this period, at which time blood was sampled and the heart and kidneys were rapidly excised. In the second experiment, rats (n=7) were administered sunitinib at the same dose for 8 days followed by an 11-day recovery period, after which they were euthanized. Six days before (baseline) and 6 days after treatment initiation, rats were housed in metabolic cages for 48 hours, the first day to acclimatize and the second day for collection of 24-hour urine for protein measurement. In the second experiment, rats were also housed in metabolic cages 1 week after treatment discontinuation. All of the experiments were performed under the regulation and permission of the animal care committee of the Erasmus MC.

**In Vitro Studies and Histology**

Hearts were rapidly excised from euthanized rats and perfused according to the Langendorff method.8 Coronary flow (CF) was measured with a flow probe (Transonic Systems). After a stabilization period of 30 minutes, baseline values of CF were obtained. Next, bolus injections (100 μL) of Tyrode buffer were applied 3 times to determine injection-induced changes in CF. Dose-response curves to bradykinin and Ang II were constructed by bolus injections, after which the maximum CF was determined by injecting sodium nitroprusside (10 mmol/L).

Next, the hearts were collected, and the heart weight was determined after removal of the atri and large vessels to determine the heart weight:body weight ratio. The apex was removed to isolate cardiomyocyte mitochondria. The ventricles were cut into 3 transversal slices and fixed in a 3.5% to 4.0% formaldehyde solution. After fixation, the slices were dehydrated and paraffin embedded. Gomori silver staining was applied to deparaffinized 5-μm-thick sections to visualize individual cardiomyocytes.10 Only transversally cut cells showing a nucleus were used to determine cardiomyocyte area.

Kidneys were collected directly after euthanization and sliced into 2-mm-thick transverse sections. After fixation in 3.5% to 4.0% formaldehyde solution for 12 hours, the slices were routinely processed to paraffin blocks, from which 2-μm-thick sections were cut and stained with Jones silverstain to allow microscopic examination.

**Cell Culture Study**

Human umbilical vein endothelial cells were obtained and cultured as described previously.11 Cells (passage 5 to 6) were seeded at 5000 cells per centimeter squared in 6-well plates and on reaching confluence exposed to 10 mmol/L of sunitinib (a concentration that has been reported to inhibit tyrosine phosphorylation of the VEGF receptor 2) or culture medium only.12 After 72 hours, medium and cells were collected and stored at −80°C for the determination of endothelin-1 (ET-1).

**Biochemical Measurements**

Plasma renin activity was measured by an in-house assay as described previously.13 Plasma renin concentration was measured by an immunoradiometric assay (Cisbio), aldosterone by radioimmunooassay (Coat-A-Count, Siemens), ET-1 by chemiluminescent ELISA (QuantiGlo, R&D Systems), VEGF by enzyme immunoassay (Quanti- tine, R&D Systems), human N-terminal probrain natriuretic peptide (NT-proBNP) by radioimmunoassay (Phoenix Pharmaceuticals, Inc), rat B-type natriuretic peptide-45 by enzyme immunoassay (Phoenix Pharmaceuticals, Inc), and total protein by colorimetric detection (Pierce Protein Assay kit). Catecholamines were measured by electrochemical detection after separation by high-performance liquid chromatography.14,15 von Willebrand factor antigen was determined with an in-house ELISA assay, using polyclonal rabbit antihuman von Willebrand factor and horseradish peroxidase–conjugated antihuman von Willebrand factor (DakoCytomation). Serum creatinine and urinary protein were measured at the clinical chemical laboratory of the Erasmus MC.

Cardiac mitochondrial respiratory activity, complex I- and II-dependent respiration, mitochondrial ATP production, and mitochondrial swelling were measured as described in the online Data Supplement, available at http://hyper.ahajournals.org.

**Data Analysis**

Data are presented as mean±SEM or geometric mean and 95% CIs. Data obtained with the Langendorff preparation were recorded and digitalized as described previously.8 Statistical analysis between groups was performed by repeated-measures ANOVA followed by Newman-Keuls multiple comparison testing or 2-way ANOVA. VEGF and NT-proBNP levels were analyzed by Wilcoxon rank-sum test. For correlation analysis, the Pearson r correlation coefficient was used. P<0.05 was considered significant. GraphPad Prism version 4.03 was used for all of the statistical analyses.

**Results**

**Clinical Study**

Fifteen patients (10 men and 5 women), mean age of 59.8±6.1 years, with metastatic renal cell carcinoma (n=13) or gastrointestinal stromal tumor (n=2) were included. Five patients had pre-existing hypertension. Twelve patients had a history of smoking, 3 were still smoking, and 3 had a history of heavy alcohol consumption.
of cardiovascular disease. Treatment with sunitinib was associated with a rise in BP showing a clear on/off effect according to the on/off treatment regimen and was accompanied by opposite changes in HR (Figure 1). Ambulatory BP measurement also showed a rise in day and night BP that sustained during the second treatment cycle (Figure 1). The rise in BP was accompanied by an attenuation of the circadian BP rhythm (Figure 1). Initiation or adjustment of antihypertensive therapy was indicated in 6 and 2 patients, respectively.

Body weight decreased from 80±3 kg at baseline to 78±4 kg (P=0.31) at week 4 and to 73±4 kg (P=0.02) at week 10. Serum creatinine (77±6 μmol/L) did not change. Proteinuria increased from 0.19±0.02 to 0.44±0.16 g/24 hours (P=0.14) at week 4, decreased to 0.20±0.04 g/24 hours (P=0.97) at week 6, and increased again to 0.35±0.13 g/24 hours (P=0.19) at week 10. Treatment with sunitinib was associated with a fall in plasma renin concentration and plasma renin activity and an increase in plasma ET-1 and VEGF concentration, whereas plasma concentrations of aldosterone, norepinephrine, epinephrine, NT-proBNP, and von Willebrand factor did not change (Table). Changes in plasma ET-1 and renin concentration did not correlate with the changes in BP.

**Rat Study**

Daily administration of sunitinib to Wistar-Kyoto rats induced a rise in BP within 1 to 2 days, reaching a plateau after 6 days. At that time, MAP had increased by ∼30 mm Hg, whereas HR had decreased (Figure 2A). The rise in BP was accompanied by an inversion of the normally occurring decrease in BP during sleep (Figure 2A). In control rats, BP

**Table. Plasma Concentrations of Neurohormones, von Willebrand Factor Antigen (vWF-Ag), and VEGF at Baseline and at the End of the First (Week 4) and Second (Week 10) Treatment Cycles of Sunitinib in Patients**

<table>
<thead>
<tr>
<th>Plasma Parameters</th>
<th>Week 0</th>
<th>Week 4</th>
<th>Week 10</th>
<th>P</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRC, pg/mL</td>
<td>9.9±2.1</td>
<td>3.6±0.8</td>
<td>5.2±1.9</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>PRA, pmol of Ang I per mL per h</td>
<td>1.3±0.3</td>
<td>0.5±0.1</td>
<td>0.75±0.2</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Aldosterone, pg/mL</td>
<td>44.5±13.1</td>
<td>36.8±8.6</td>
<td>55.2±16.6</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>ET-1, pg/mL</td>
<td>3.0±0.4</td>
<td>5.2±0.8</td>
<td>6.0±1.2</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Norepinephrine, pg/mL</td>
<td>265.5±23.3</td>
<td>258.3±37.4</td>
<td>351.3±83.5</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Epinephrine, pg/mL</td>
<td>31.7±8.9</td>
<td>25.8±4.9</td>
<td>41.2±14.7</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>vWF-Ag, U/mL</td>
<td>1.6±0.2</td>
<td>1.7±0.2</td>
<td>1.4±0.2</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>NT-proBNP, pg/mL</td>
<td>850.5 (648.1 to 1116.0)</td>
<td>885.6 (637.4 to 1230.0)</td>
<td>729.2 (521.2 to 1020.0)</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>VEGF, pg/mL</td>
<td>35.5 (22.9 to 55.2)</td>
<td>153.6 (83.9 to 280.9)</td>
<td>150.6 (63.0 to 360.1)</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>

PRC indicates plasma renin concentration; PRA, plasma renin activity. Data are shown as mean±SEM. VEGF and NT-proBNP values are shown as geometric mean and 95% CIs. P value is compared with baseline (week 0).
and HR remained stable (Figure 2A). Both the rise in BP and the attenuation of its circadian rhythm were reversible after sunitinib withdrawal (Figure 2B).

The increase in body weight (from 368±3 to 382±3 g) was lower (P<0.001) in rats on sunitinib than in control rats (from 383±3 to 406±3 g). Serum creatinine increased during administration of sunitinib and remained elevated after its discontinuation (Figure 3A). Administration of sunitinib was associated with proteinuria that normalized after sunitinib withdrawal (Figure 3B). Histological evaluation of the kidney using light microscopy showed marked glomerular changes after sunitinib treatment (Figure S1, please see the online Data Supplement at http://hyper.ahajournals.org). Comparable to findings in patients, administration of sunitinib was associated with a 3-fold increase in plasma ET-1 concentration, which normalized after its discontinuation (Figure 3C).

Circulating B-type natriuretic peptide 45 levels were 2-fold higher in the sunitinib-administered group compared with the control group (164±34 versus 93±15 ng/mL; P=0.03) and normalized after sunitinib withdrawal (93±9 ng/mL; P=0.03). Heart weight:body weight ratio (3.00±0.04 versus 3.10±0.06 g/kg; P=0.18) and cardiomyocyte area (513±37 versus 552±42 µm²; P=0.51) were not different between the sunitinib and control groups.

In the Langendorff preparation, CF responses to bradykinin, Ang II, and sodium nitroprusside were markedly attenuated in sunitinib-administered rats (Figure 4; P=0.05), and all of the responses normalized after discontinuation of sunitinib. Complex I- and II–dependent basal respiratory activity (state 2), as well as the ADP-stimulated (state 3) respiration, were not different between groups (Figure S2A and S2B). The respiratory control indices did not change either (data not shown). Complex I- and II–dependent ATP productions were not different in the sunitinib compared with the control group or after sunitinib withdrawal (Figure S2C). The calcium-induced mitochondrial swelling was lower in the sunitinib than in the control group and remained low after sunitinib withdrawal (Figure S2D).

Figure 2. A, Changes in MAP, HR, and dipping of MAP during sleep (day 6) in response to sunitinib (n=10) or vehicle treatment in rats (n=6). B, Changes in MAP and HR during administration and after discontinuation of sunitinib (n=7). MC indicates metabolic cage. *P<0.05.

Figure 3. Serum creatinine (A), proteinuria (B), and serum ET-1 (C) in rats after administration of sunitinib (n=10 to 13), vehicle (control; n=6), or sunitinib withdrawal (stop; n=7). *P<0.05 vs baseline/control.
tension in pregnant rats during infusion of soluble fms-like tyrosine kinase 1, a VEGF binding factor, is involved in the pathogenesis of preeclampsia. Indeed, accumulating evidence that increased placental production of fms-like tyrosine kinase 1 impairs VEGF signaling, its function did not. Third, in our clinical study the BP elevation in pregnant rats during infusion of soluble fms-like tyrosine kinase 1 for 6 days. Such infusion was accompanied by a 3-fold increased expression of preproendothelin mRNA in the renal cortex but not by increased expression in the renal medulla or aorta. Endothelin A receptor blockade in this model completely prevented the BP rise. Because soluble fms-like tyrosine kinase 1 impairs VEGF signaling, its infusion may, therefore, create a condition comparable to that induced by sunitinib, and the increased ET-1 levels in our studies might be of renal origin as well.

Because the rise in BP was associated with a decrease in renin, involvement of the renin-Ang system could be excluded as an underlying mechanism. Despite the decrease in renin, aldosterone levels did not change. The possibility that mineralocorticoid-receptor activation has played a role in the development of hypertension can, therefore, not be excluded. The sunitinib-induced rise in BP was not associated with an increase in plasma catecholamine levels, indicating that sympathetic nervous system activation was not involved in the BP elevation. This is supported by the observation that the rise in BP was accompanied by a decrease in HR. To some extent, our findings disagree with those reported by Veronese et al., because these authors did not report an increase in plasma ET-1 concentration or a decrease in plasma renin activity during treatment with the RTKI sorafenib. Sorafenib has partly different receptor targets compared with sunitinib, which might account for the different findings in both studies. Furthermore, in the study of Veronese et al., baseline blood samples were taken in the first 2 weeks after treatment initiation, and changes in hormone levels might therefore have been missed.

Renal function impairment, apart from the development of hypertension, is also a well-documented adverse effect of angiogenesis inhibition. In our clinical study, no change in serum creatinine concentration was observed, but proteinuria tended to increase. In contrast, in our experimental study sunitinib was associated with a marked impairment of renal function, development of pronounced proteinuria, and glomerular changes. For several reasons it is unlikely that impairment of renal function accounted for the rise in BP. First, and in accordance with other clinical and experimental studies, the rise in BP already occurred within 1 to 2 days after administration of sunitinib. Second, after sunitinib withdrawal in rats, BP completely normalized, whereas renal function did not. Third, in our clinical study the BP elevation.

**Cell Culture Study**

ET-1 production by sunitinib-treated human umbilical vein endothelial cells was identical to that in control cells (22±5 versus 20±5 ng/µg of protein; P=0.73; n=9).

**Discussion**

Hypertension is a frequent and sometimes severe adverse effect of antiangiogenic therapy. With our clinical and experimental studies we aimed to obtain more insight into the mechanisms underlying the development of hypertension. In agreement with previous reports we found that the RTKI sunitinib induced a substantial rise in BP. Evaluation of the time course of the BP rise in our experimental study revealed that this already occurred 1 day after its administration. Furthermore, both in humans and in rats the BP response showed an on/off effect that paralleled the on/off administration regimen. In patients the rise in BP was accompanied by attenuation and in rats by reversal of the circadian BP variation, and in both species it was accompanied by a decline in HR. Remarkably, both in our clinical and experimental studies, sunitinib administration was associated with a substantial rise in circulating ET-1 levels. To our knowledge, increased plasma ET-1 levels in response to sunitinib have not been reported before. Although ET-1 is predominantly produced by endothelial cells, sunitinib did not affect ET-1 release from human umbilical vein endothelial cells. This suggests that the ET-1 increase has a nonendothelial origin.

The question of whether activation of the endothelin system is involved in the sunitinib-induced rise in BP cannot be answered at this moment. In a recent study performed in telemetry-instrumented rats, pretreatment with the selective endothelin A receptor antagonist atrasentan completely prevented the rise in BP induced by a RTKI. Because the same endothelin A receptor blocker also lowered BP in control rats, definite conclusions about the role of the endothelin system, in particular, activation of endothelin A receptors, in the rise of BP associated with the use of antiangiogenic therapy cannot be drawn. Notably, increased plasma ET-1 levels have also been described in patients with preeclampsia. There is accumulating evidence that increased placental production of soluble fms-like tyrosine kinase 1, a VEGF binding factor, is involved in the pathogenesis of preeclampsia. Indeed, recently Murphy et al reported the development of hypertension in pregnant rats during infusion of soluble fms-like tyrosine kinase 1 for 6 days. Such infusion was accompanied by a 3-fold increased expression of preproendothelin mRNA in the renal cortex but not by increased expression in the renal medulla or aorta. Endothelin A receptor blockade in this model completely prevented the BP rise. Because soluble fms-like tyrosine kinase 1 impairs VEGF signaling, its infusion may, therefore, create a condition comparable to that induced by sunitinib, and the increased ET-1 levels in our studies might be of renal origin as well.

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**Figure 4.** CF responses to bradykinin (BK; n=6 to 10; A), Ang II (n=7 to 9; B), and a single injection of sodium nitroprusside (SNP; 10 mmol/L; n=6 to 10; C) in isolated rat hearts after administration of sunitinib, vehicle (control), or sunitinib withdrawal (stop). The x axis in A and B displays the concentration in the injection fluid. T indicates Tyrode’s buffer. *P<0.05 vs control.
was not accompanied by any deterioration in renal function. Because body weight or NT-proBNP levels did not increase in our patients, fluid retention is also unlikely to be involved in the rise in BP.

VEGF has been reported to increase the expression of endothelial NO synthase. Inhibition of VEGF signaling may, therefore, result in impaired NO production, leading to hypertension.\(^2,3\) We used the Langendorff model to investigate whether sunitinib administration impaired endothelium-dependent vasodilation. We found that not only the response to the endothelium-dependent vasodilator bradykinin, but also the responses to the endothelium-independent vasodilator sodium nitroprusside, as well as the vasoconstrictor Ang II, were considerably impaired in sunitinib-administered rats. These findings indicate that exposure to sunitinib is associated with a generalized impaired function of the vascular smooth muscle cell. From our studies we cannot conclude whether this is related to inhibition of VEGF signaling and/or interaction of sunitinib with other target receptors, or a consequence of the elevated BP.

Not only hypertension but also left ventricular dysfunction in patients and cardiomyocyte hypertrophy and changes in mitochondrial structure in endomyocardial biopsy samples of patients have been observed during sunitinib treatment.\(^5\) Impaired ATP generation secondary to mitochondrial dys-function has been proposed as an underlying mechanism for the development of cardiac dysfunction.\(^27\) However, studies performed in isolated rat heart mitochondria have shown that sunitinib, contrary to sorafenib, impairs mitochondrial function only at supratherapeutic concentrations.\(^6\) In accordance with these findings in our ex vivo studies no evidence was found that sunitinib administration, at a dose that markedly increased BP, affected mitochondrial ATP production. Because of the large interanimal variation in cardiac mitochondrial ATP production and the necessity to perform the studies in different groups of animals, we cannot completely exclude the possibility that an effect of sunitinib on ATP production has been missed. In our current opinion the observed increase in plasma B-type natriuretic peptide levels in the rats is more likely a consequence of elevated BP, as well as a decreased renal clearance, rather than reflecting direct cardiac damage by sunitinib.

Some limitations of our studies should be addressed. First, we did not perform a randomized, controlled trial. Because sunitinib is a first-line treatment of patients with metastatic renal cell carcinoma or imatinib-resistant gastrointestinal stromal tumor it is not possible to perform a placebo-controlled trial in these patients. Second, the number of included patients in our study was rather limited. Despite this limited number we still found relevant changes in BP and various neurohormones because the responses of these parameters among patients were quite uniform. Third, because of our extensive experience with this cell line, we have used human umbilical vein endothelial cells to investigate the effect of sunitinib on ET-1 release. Because no increased ET-1 release in response to sunitinib could be demonstrated, it would be worthwhile to study the effect of sunitinib on ET-1 release in other endothelial cell lines to ascertain the uniformity of this finding.

**Perspectives**

Inhibition of VEGF signaling with the multitarget RTKI sunitinib is associated with a substantial rise in BP according to an on/off mechanism in both humans and rats and is accompanied by a rise in circulating ET-1 concentration. Whether this activation of the endothelin system is instrumental for the rise in BP cannot be concluded at this moment and requires further investigation. Experimental evidence that the endothelin system plays a role in the development of hypertension during administration of antiangiogenic agents is emerging.\(^17,21\) If such a mechanism can also be established in patients, antihypertensive treatment with an endothelin receptor blocker would be a logical choice. Finally, in our clinical study the development of hypertension was associated with renin suppression. The implication of this finding could be that agents interfering with the renin-Ang system are less effective in reducing BP in antiangiogenic therapy-induced hypertension and that calcium channel blockers or, as discussed earlier, endothelin receptor blockers may be preferable instead. Because the use of antiangiogenic agents is expected to increase, future clinical and experimental studies have to be performed to establish which antihypertensive therapy is most effective for the management of hypertension associated with these drugs.

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**Disclosures**

None.

**References**


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HYPERTENSION INDUCED BY THE TYROSINE KINASE INHIBITOR SUNITINIB IS ASSOCIATED WITH INCREASED CIRCULATING ENDOTHELIN-1 LEVELS

Mariëtte H.W. Kappers, MD; Joep H.M. van Esch, PhD; Wim Sluiter¹, PhD;
Stefan Sleijfer², MD, PhD; A.H. Jan Danser, PhD; Anton H. van den Meiracker, MD, PhD

Division of Pharmacology, Vascular and Metabolic Diseases,
Department of Internal Medicine, Department of Neurology¹, Department of Medical Oncology², Erasmus MC, Rotterdam,
The Netherlands

Correspondence to:
M.H.W. Kappers, MD
Division of Pharmacology, Vascular and Metabolic Diseases
Department of Internal Medicine
Erasmus MC
‘s Gravendijkwal 230 P.O. Box 2040
3015 CE Rotterdam 3000 CA Rotterdam
The Netherlands The Netherlands
Tel: +31107032196
Fax: +31104366868
Email: m.h.w.kappers@erasmusmc.nl

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Expanded methods

Cardiac mitochondrial function assessment
Mitochondrial respiratory activity, measured as the oxygen consumption rate (flux in pmol oxygen/second/mg mitochondrial protein) was assessed at 37°C by high-resolution respirometry (Oxygraph-2k, Oroboros Instruments). Complex I- and II-dependent respiration were measured in state 2 (respectively, in the presence of 2 mmol/L malate and 10 mmol/L glutamate, and in the presence of 10 mmol/L succinate) and state 3 (in the presence of substrates and 0.25 mmol/L ADP).1 To prevent retrograde flux of electrons via complex I, complex II-dependent respiration was measured in the presence of 0.5 μmol/L of the complex I inhibitor rotenone. The respiratory adenylate control index (RCI) was calculated by dividing the oxygen flux in state 3 by the flux in state 2. Mitochondrial ATP production was determined by incubating mitochondrial protein (1, 10 and 100 ng/mL) in medium as described by Korsten et al, but without digitonin, in 96-well black microplates (Optiplate; PerkinElmer).2 Mitochondrial swelling was measured as described by Wang et al.3 Pore opening was induced by 2 μmol/L CaCl_2 with or without 30 nmol/L cyclosporine A. Swelling was measured as a change in light (520 nm) absorption/min 5 minutes after addition of CaCl_2.

References
2. Korsten A, de Coo IF, Spruijt L, de Wit LE, Smeets HJ, Sluiter W. Patients with leber hereditary optic neuropathy fail to compensate impaired oxidative phosphorylation. Biochim Biophys Acta. 2010;1797:197-203
Figure S1.
Kidney sections from WKY-rats treated with vehicle (A) and sunitinib (B) stained with Jones silverstaining (magnification ×800). The glomerulus of the vehicle-treated rat is nicely unfolded showing wide capillary lumina, filled with numerous erythrocytes, normal-sized endothelial cells (white arrowhead) and epithelial cells (arrow). After administration of sunitinib, the glomerulus appears more shrinked with narrowing of the capillary lumina and swelling of endothelial (arrowhead) and epithelial cells (arrow).
Figure S2.
Complex I-dependent (panel A), complex II-dependent (panel B) state 2 and state 3 mitochondrial respiratory activity (measured as oxygen consumption or O2-flux), ATP production (panel C) and swelling (panel D) in mitochondria isolated from cardiomyocytes of rats after administration of sunitinib (n=6-13), vehicle (control; n=7) or sunitinib withdrawal (stop; n=7). *P<0.05 compared to control.