Sunitinib-Induced Systemic Vasoconstriction in Swine Is Endothelin Mediated and Does Not Involve Nitric Oxide or Oxidative Stress


Abstract—Angiogenesis inhibition with agents targeting tyrosine kinases of vascular endothelial growth factor receptors is an established anticancer treatment, but is, unfortunately, frequently accompanied by systemic hypertension and cardiac toxicity. Whether vascular endothelial growth factor receptor antagonism also has adverse effects on the pulmonary and coronary circulations is presently unknown. In chronically instrumented awake swine, the effects of the vascular endothelial growth factor receptor antagonist sunitinib on the systemic, pulmonary, and coronary circulation were studied. One week after sunitinib (50 mg PO daily), mean aortic blood pressure (MABP) had increased from 83±5 mm Hg at baseline to 97±6 mm Hg (P<0.05) because of a 57±20% increase in systemic vascular resistance as cardiac output decreased. In contrast, sunitinib had no discernible effects on pulmonary and coronary hemodynamics or cardiac function. We subsequently investigated the mechanisms underlying the sunitinib-induced systemic hypertension. Intravenous administration of NO synthase inhibitor N^6^-nitro-L-arginine increased MABP by 24±1 mm Hg under baseline conditions, whereas it increased MABP even further after sunitinib administration (32±3 mm Hg; P<0.05). Reactive oxygen species scavenging with a mixture of antioxidants lowered MABP by 13±2 mm Hg before but only by 5±2 mm Hg (P<0.05) after sunitinib administration. However, intravenous administration of the dual endothelin A/endothelin B receptor blocker tezosentan, which did not lower MABP at baseline, completely reversed MABP to presunitinib values. These findings indicate that sunitinib produces vasoconstriction selectively in the systemic vascular bed, without affecting pulmonary or coronary circulations. The sunitinib-mediated systemic hypertension is principally attributed to an increased vasoconstrictor influence of endothelin, with no apparent contributions of a loss of NO bioavailability or increased oxidative stress. (Hypertension. 2012;59:151-157.)

Key Words: endothelin 1 ■ hypertension ■ oxidative stress ■ pulmonary hypertension ■ vascular endothelial growth factor

Angiogenesis inhibition, by targeting the tyrosine kinases of the vascular endothelial growth factor (VEGF) receptors (VEGFRs), has become an established treatment of several tumor types. This therapy is associated with adverse effects, including the development of hypertension and cardiac and renal toxicity. Hyper trophy has been reported in ≥60% of patients treated with sunitinib, an orally active multitarget receptor tyrosine kinase inhibitor (RTKI), targeting, among others, the VEGFR-1 and -2, which is used as first-line treatment of metastatic renal cell carcinoma or imatinib-resistant gastrointestinal stromal tumors. In addition, impaired cardiac function, as reflected by a decrease in left ventricular ejection fraction of 10% to 15%, has been observed in ≥28% of patients treated with sunitinib. Angina pectoris and increased levels of biomarkers reflecting ischemic myocardial damage may also occur during sunitinib treatment. Thus far, clinical and experimental studies on the cardiovascular adverse effects of angiogenesis inhibition only focused on the systemic vasculature. Whether adverse effects of angiogenesis inhibition with sunitinib also occur in the pulmonary and/or coronary circulation is unknown. It should be mentioned that pulmonary arterial hypertension has been reported recently during treatment with dasatinib, an RTKI used for the treatment of chronic myeloid leukemia.

VEGF, through activation of VEGFR-2, stimulates endothelial NO synthase (eNOS), resulting in enhanced NO production and vasodilation. It has, therefore, been suggested that NO bioavailability is reduced during inhibition of the VEGF pathway, resulting in vasoconstriction and the development of hypertension. Indeed, in mice, administration...
of an anti–VEGFR-2 antibody, targeting the same receptor as RTKIs, caused a rapid increase in mean aortic blood pressure (MABP) and a marked reduction in the expression of endothelial and neuronal NO synthase in the kidney.7 Furthermore, administration of the eNOS inhibitor N\textsubscript{G}-nitro-L-arginine methyl ester in mice abolished the difference in MABP between vehicle and anti–VEGFR-2–treated groups, suggesting that decreased NO bioavailability in response to antiangiogenic agents is one of the mechanisms causing hypertension.7 However, clinical studies, using flow-mediated dilatation (FMD) as an index of NO bioavailability, do not unequivocally support the hypothesis that a decrease in NO bioavailability underlies the increase in MABP, because decreases in both endothelium-dependent and endothelium-independent vasodilatation have been reported in patients treated with RTKIs.8,9 In previous clinical and experimental studies, we and others have shown that activation of the endothelin (ET) system is involved in the sunitinib-induced rise in MABP.10–12 Apart from inducing vasoconstriction, ET-1 activates vascular NADPH oxidase, leading to an increase in oxidative stress through enhanced reactive oxygen species (ROS) production.13,14 Because oxidative stress is generally increased in hypertension and plays a pathogenic role in the development and progression of cardiovascular disease, we hypothesized that enhanced formation of ROS contributes to the sunitinib-induced cardiovascular adverse effects.15

To further explore the cardiovascular adverse effects of sunitinib, we performed detailed studies in chronically instrumented awake swine. First, we studied the effects of angiogenesis inhibition on the systemic, pulmonary, and coronary circulations, as well as on cardiac performance under resting conditions and during exercise. Second, we explored to what extent alterations in NO-mediated vasodilator tone, ET-1–mediated vasoconstriction, and oxidative stress contribute to the cardiovascular adverse effects of angiogenesis inhibition.

Methods

Animals

Studies were performed in accordance with the American Physiological Society Guiding Principles in the Care and Use of Laboratory Animals and with approval of the Animal Care Committee of Erasmus MC. Six crossbred Yorkshire × Landrace swine of either sex (2–3 months old; 21±1 kg at the time of surgery) were entered into the study.

Surgical Procedures

Swine were sedated (20 mg/kg of ketamine IM + 1 mg/kg midazolam IM), anesthetized (tiopental sodium, 15 mg/kg IV), intubated, and ventilated with a mixture of O\textsubscript{2} and N\textsubscript{2} (1:2).16 Anesthesia was maintained with midazolam (2 mg/kg + 1 mg · kg\textsuperscript{-1} · h\textsuperscript{-1} · IV) and fentanyl (10 μg · kg\textsuperscript{-1} · h\textsuperscript{-1} · IV). Subsequently, animals were instrumented under sterile conditions as described previously.17,18 Briefly, a thoracotomy was performed through the left fourth intercostal space. Subsequently, a polyvinylchloride catheter was inserted into the aortic arch, for the measurement of aortic pressure and blood sampling for the determination of Po\textsubscript{2}, PCO\textsubscript{2}, pH, O\textsubscript{2} saturation, and hemoglobin concentration (ABL800, Radiometer). A high-fidelity Konigsberg pressure transducer was inserted into the left ventricle (LV) via the apex for measurement of LV pressure and maximum rate of rise and fall of LV pressure (LVdp/dt\textsubscript{max}, LVdp/ dt\textsubscript{min}) as indices for contractility and relaxation. Fluid-filled catheters were implanted in the LV, left atrium, and pulmonary artery for measurement of blood pressures and blood sampling. Flow probes were placed around the proximal left anterior descending coronary artery (2.5–3.0 mm, Transonic Systems) for measurement of coronary blood flow (CBF) and around the aorta (16 mm, Transonic Systems) for measurement of cardiac output and stroke volume (SV).16–18 Electric wires and catheters were tunneled subcutaneously to the back. The chest was closed and the animals were allowed to recover. Animals received analgesia (0.3 mg of buprenorphine IM) for 2 days and antibiotic prophylaxis (25 mg/kg of amoxicillin and 5 mg/kg of gentamicin IV) for 5 days.

Sunitinib

Studies were performed 1 to 4 weeks after surgery with swine resting and/or exercising on a motor-driven treadmill. Excellent reproducibility of exercise trials has been reported previously.17,18 After obtaining hemodynamic measurements with swine lying quietly on a treadmill, a 5-stage (1–5 km/h) treadmill exercise protocol was started with each exercise stage lasting 2 to 5 minutes. Hemodynamic variables were continuously recorded, and both arterial and mixed venous blood samples were collected during the last 60 s of each exercise stage for the determination of body oxygen consumption.18 Sunitinib 1-malate was given in a daily oral dose of 50 mg, the same dose as used in patients. Sunitinib was mixed with food and administered early in the morning. Resting hemodynamics were measured 4 hours after the first dose of sunitinib and after 1 week of daily sunitinib administration. After 1 week of sunitinib administration, the exercise protocol was repeated to assess the effects of sunitinib on exercise-induced hemodynamic responses.

Acute Pharmacological Interventions

After measuring systemic, pulmonary, and coronary hemodynamics at rest, acute intravenous doses of an eNOS inhibitor, a dual ET\textsubscript{A} and ET\textsubscript{B} receptor (ET\textsubscript{A}/ET\textsubscript{B}) antagonist or an ROS scavenger mixture were given on separate days in random order and cardiovascular effects were assessed. Subsequently swine were exposed to sunitinib for 7 to 10 days, and the pharmacological interventions were repeated, in random order on separate days, to assess the roles of NO, ET-1, and ROS in sunitinib-induced cardiovascular effects.

Pharmacological Agents

The eNOS inhibitor N\textsubscript{G}-nitro-L-arginine (LNNA; Sigma) was given as a single IV dose of 20 mg/kg.19 The dual ET\textsubscript{A} and ET\textsubscript{B} receptor (ET\textsubscript{A}/ET\textsubscript{B}) antagonist tezosentan (a kind gift from Dr Martine Clozel, Actelion Pharmaceuticals Ltd) was intravenously administered over 10 minutes in a dose of 3 mg/kg, followed by a continuous infusion of 6 mg · kg\textsuperscript{-1} · h\textsuperscript{-1} · IV.20 For ROS scavenging, the superoxide dismutase-mimetic Tempol (30 mg/kg IV), in combination with N-acetyl-cysteine (150 mg/kg IV), and N-mercapto-propionylglycine (1 mg/kg/min IV) were infused in 10 minutes before the measurements.21–24

Determination of ET

ET was assessed in plasma from 4 swine, obtained before and after sunitinib, using a chemiluminescent ELISA according to the manufacturer’s protocol (QuantiGlo, R&D Systems).

Data Acquisition and Analysis

Digital recording and offline analysis of hemodynamic data and computation of body O\textsubscript{2} consumption have been described in detail elsewhere.16,18 To correct for growth of swine, cardiac index (CI) and stroke volume index (SVI) were calculated as cardiac output and stroke volume, respectively, divided by body weight. Systemic vascular resistance index (SVRI) was computed as MABP divided by CI. Pulmonary vascular resistance index (PVRI) was computed as mean pulmonary artery pressure minus mean left atrial pressure divided by CI. Finally, coronary vascular resistance (CVR) was calculated as MABP divided by CBF. Statistical analysis was performed using regression analysis with each animal as a dummy variable and with body O\textsubscript{2} consumption and rate-pressure product (calculated as heart rate × LV systolic pressure), as well as sunitinib, as independent variables. Statistical significance was accepted at P≤0.05. Data are presented as mean±SEM.
Results

Effect of Sunitinib at Rest
Oral administration of the first dose of sunitinib resulted in an increase in MABP within 4 hours in 5 of 6 animals but did not result in a change in plasma ET levels (Table 1). The increase in MABP was associated with an increase in LV systolic pressure, but LVdP/dt\textsubscript{max}, LVdP/dt\textsubscript{min}, and SV\textsubscript{i} did not change (Table 1). Also, sunitinib had no effect on the coronary or pulmonary vasculature, because CBF, CVR, pulmonary artery pressure, and PVR\textsubscript{i} were similar to values before sunitinib administration (Table 1).

Seven days of daily sunitinib administration resulted in sustained systemic vasoconstriction, as evidenced by an increase in SV\textsubscript{R}\textsubscript{i} and MABP in all of the animals (Table 1). The increase in MABP was accompanied by a decrease in heart rate and CI and an increase in LV systolic pressure, whereas LVdP/dt\textsubscript{max}, LVdP/dt\textsubscript{min}, and SV\textsubscript{i} were unchanged. Also, no change in CBF occurred, whereas CVR slightly increased, likely reflecting an autoregulatory increase in coronary vasomotor tone in response to the increase in MABP. Repeated doses of sunitinib had no effect on the pulmonary vasculature, because mean pulmonary artery pressure and PVR\textsubscript{i} were similar to values before sunitinib administration (Table 1). Moreover, plasma ET levels were not significantly altered after 1 week of sunitinib (Table 1).

Effect of Sunitinib on Hemodynamic Parameters During Exercise
Sunitinib did not influence the exercise-induced increase in heart rate or CI, whereas the sunitinib-induced increase in MABP and SV\textsubscript{R}\textsubscript{i} decreased with increasing exercise intensity (Figure 1), indicating that the vasoconstrictor effect of sunitinib diminished with increasing exercise intensity. Sunitinib also had no effect on mean pulmonary artery pressure and PVR\textsubscript{i} during exercise, whereas the sunitinib-induced increase in CVR decreased with exercise intensity (Figure 1), reflecting the waning effect of sunitinib on MABP during exercise.

Table 1. Resting Hemodynamic Variables Before and 4 h and 7 d After Sunitinib Administration

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>4 h Sunitinib</th>
<th>7 d Sunitinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>112±6</td>
<td>111±10</td>
<td>105±7*</td>
</tr>
<tr>
<td>MABP, mm Hg</td>
<td>83±5</td>
<td>93±5*</td>
<td>97±6*</td>
</tr>
<tr>
<td>CI, mL \cdot min\textsuperscript{-1} \cdot kg\textsuperscript{-1}</td>
<td>155±11</td>
<td>162±17</td>
<td>132±12*</td>
</tr>
<tr>
<td>SVR\textsubscript{i}, mm Hg \cdot L\textsuperscript{-1} \cdot min\textsuperscript{-1}</td>
<td>547±45</td>
<td>607±69</td>
<td>780±108*</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>110±6</td>
<td>115±8</td>
<td>116±10</td>
</tr>
<tr>
<td>SIV, mL \cdot kg\textsuperscript{-1}</td>
<td>1.38±0.06</td>
<td>1.46±0.06*</td>
<td>1.26±0.10</td>
</tr>
<tr>
<td>LV dP/dt\textsubscript{max}, mm Hg \cdot s\textsuperscript{-1}</td>
<td>2800±460</td>
<td>2870±260</td>
<td>2620±250</td>
</tr>
<tr>
<td>LV dP/dt\textsubscript{min}, mm Hg \cdot s\textsuperscript{-1}</td>
<td>-2220±160</td>
<td>-2280±130</td>
<td>-2320±190</td>
</tr>
<tr>
<td>MPAP, mm Hg</td>
<td>15±2</td>
<td>17±2</td>
<td>14±2</td>
</tr>
<tr>
<td>MLAP, mm Hg</td>
<td>5±2</td>
<td>7±2</td>
<td>4±1</td>
</tr>
<tr>
<td>PVR\textsubscript{i}, mm Hg \cdot L\textsuperscript{-1} \cdot min\textsuperscript{-1}</td>
<td>64±12</td>
<td>59±5</td>
<td>68±6</td>
</tr>
<tr>
<td>CBF, mL/min</td>
<td>48±2</td>
<td>44±2</td>
<td>47±3</td>
</tr>
<tr>
<td>CVR, mm Hg \cdot mL\textsuperscript{-1} \cdot min\textsuperscript{-1}</td>
<td>1.64±0.14</td>
<td>1.90±0.23</td>
<td>2.00±0.17*</td>
</tr>
<tr>
<td>ET, pg/mL</td>
<td>2.4±0.6</td>
<td>2.5±0.7</td>
<td>2.8±1.0</td>
</tr>
</tbody>
</table>

*Values are mean±SE. HR indicates heart rate; MABP, mean aortic blood pressure; CI, cardiac index; SVR\textsubscript{i}, systemic vascular resistance index; LVSP, left ventricular systolic pressure; SIV, stroke volume index; LV dP/dt, left ventricular rate of change in pressure; MPAP, mean pulmonary artery pressure; MLAP, mean left atrial pressure; PVR\textsubscript{i}, pulmonary vascular resistance index; CBF, coronary blood flow; CVR, coronary vascular resistance; ET, plasma endothelin levels. *P<0.05 vs baseline.

Figure 1. Effect of sunitinib on systemic, pulmonary, and coronary hemodynamic parameters at rest and during graded treadmill exercise. BVO\textsubscript{2} indicates body oxygen consumption; MABP, mean aortic blood pressure; SVR\textsubscript{i}, systemic vascular resistance; MPAP, mean pulmonary artery pressure; PVR\textsubscript{i}, pulmonary vascular resistance; RPP, rate pressure product; CBF, coronary blood flow; CVR, coronary vascular resistance. *P<0.05 sunitinib vs control.
Effect of Inhibition of NO, ET, or ROS on Hemodynamic Parameters During Sunitinib

Administration of LNNA resulted in systemic vasoconstriction, as evidenced by an increase in SVRi that resulted in increases in MABP and LV systolic pressure (Figure 2). The increase in MABP was associated with a likely baroreflex-mediated decrease in heart rate and CI (Table 2). The effect of LNNA on systemic hemodynamics was slightly more pronounced after 1 week of sunitinib treatment (Figure 2). Under baseline conditions, LNNA also increased PVRi, reflecting pulmonary vasoconstriction, whereas the effect of LNNA on PVRi after sunitinib treatment failed to reach statistical significance (P = 0.13; Figure 2). LNNA had no effect on CBF, but CVR increased. Sunitinib did not affect the LNNA-induced increase in CVR (Figure 2).

Contrary to previous findings from our laboratory, in which ET<sub>A</sub>/ET<sub>B</sub> receptor blockade with tezosentan resulted in a slight decrease in MABP, tezosentan had no effect on MABP or SVRi before sunitinib in the present study (Figure 2). In contrast, in the presence of sunitinib, MABP and SVRi decreased in response to tezosentan administration (Figure 2). The tezosentan-induced decrease in SVRi was identical to the increase in SVRi induced by sunitinib. In the absence of sunitinib, administration of tezosentan resulted in a decrease in PVRi. This tezosentan-induced pulmonary vasodilation was not altered by administration of sunitinib (Figure 2). Tezosentan had no effect on the coronary vasculature (Figure 2) or on LV systolic pressure, LVdP/dt<sub>min</sub>, and SVi in the absence of sunitinib, whereas tezosentan slightly increased SVi and dP/dt<sub>max</sub> after 1 week of sunitinib administration (Table 2).

Before sunitinib, ROS scavenging resulted in systemic vasodilation, as evidenced by the decreases in MABP and SVRi (Figure 2). The effect of ROS scavenging on MABP but not SVRi was reduced after sunitinib (Figure 2). In the pulmonary circulation, ROS scavenging resulted in a decrease in pulmonary artery pressure before sunitinib, whereas it had no effect on pulmonary artery pressure in the presence of sunitinib. Moreover, ROS scavenging did not affect PVRi either in the absence or presence of sunitinib (Figure 2). ROS scavenging had no effect on CBF either before or after sunitinib. Finally, ROS scavenging did not affect dP/dt<sub>max</sub>, dP/dt<sub>min</sub>, or SVi either before or after sunitinib (Table 2).

Discussion

The main findings of the present study in chronically instrumented awake swine are as follows: (1) administration of the RTKI sunitinib induces a rapid rise in systemic BP because of an increase in systemic vascular resistance, without affecting the pulmonary or coronary circulation; (2) the vasoconstrictor effect of sunitinib on the systemic circulation waned with increasing exercise intensities; (3) sunitinib had no adverse effects on cardiac function either at rest or during exercise; (4) the systemic hemodynamic effects of sunitinib were fully reversed on administration of the ETA/ETB receptor blocker tezosentan, confirming previous findings that an increase in endogenous ET-mediated vasoconstrictor tone is involved in the sunitinib-induced rise in MABP; and, finally, (5) no evidence was found that a decrease in NO bioavailability or an increase in oxidative stress contributed to the systemic vasoconstriction produced by sunitinib.10–12

In accordance with data in both rats and patients, administration of sunitinib induced a rapid and sustained increase in systemic MABP in swine.10–12 Because VEGF is not only important for the formation but also for the remodeling of
blood vessels, a sunitinib-induced decrease in microvessel density per unit of volume has been proposed as a potential mechanism underlying the MABP rise during antiangiogenic therapy. Indeed, prolonged treatment with the RTKIs sunitinib and telatinib has been reported to be associated with capillary rarefaction. To increase vascular resistance in a particular vascular bed by 5%, it has been estimated that 40% rarefaction of fourth-order vessels is required. It is inconceivable that such an extensive degree of systemic rarefaction can occur within 4 hours after initiation of angiogenesis inhibition. In addition, the current observations that the sunitinib-induced systemic vasoconstriction decreased with increasing levels of exercise and the immediate normalization of MABP and systemic vascular resistance with subsequent ETA/ETB blockade further argue against rarefaction as a mechanism underlying the MABP rise associated with angiogenesis inhibition (Figure 2). Therefore, we consider vasoconstriction the most important mechanism involved in the MABP rise during antiangiogenesis therapy.

To explore potential mechanisms underlying the sunitinib-induced rise in vascular resistance and MABP, we investigated the acute effects of ETA/ETB receptor blockade, eNOS inhibition, and ROS scavenging before and after 1 week of sunitinib treatment. We have shown previously that administration of sunitinib in patients and rats is associated with an increase in circulating ET-1 levels. In the present study, sunitinib did not significantly alter plasma ET levels, although an increase in plasma ET was observed in 3 of 4 animals. Because ET secretion is predominantly abluminal, its plasma level does not adequately reflect its effect on vascular tone. This effect should, therefore, preferentially be assessed using an ET receptor antagonist, as performed in the present study. We and others have demonstrated in rats that coadministration of an ET-1 receptor antagonist with an RTKI can largely or completely prevent the rise in MABP, suggesting that activation of the ET pathway plays an important role in the sunitinib-induced MABP rise. These observations in rats are fully supported by the present findings in swine, because the sunitinib-induced increase in SVRi and MABP completely reversed to presunitinib values in response to acute administration of the ET_A/ET_B receptor blocker tezosentan.

ET-1 exerts part of its vasoconstrictor effect through activation of NADPH oxidase and generation of ROS. In addition, hypertension has been reported to be associated with an increase in ROS. Because ROS can be derived from multiple sources within the circulation, including NADPH oxidase, mitochondrial respiration, and neutrophils, we used a mixture of antioxidants to achieve extensive ROS scavenging. Using this approach, the present study shows that the effect of ROS scavenging on MABP was reduced, whereas the effect on SVRi was unchanged after 1 week of sunitinib treatment. The apparent discrepancy between the effect of ROS scavenging on MABP and SVRi is most likely attributed to the reduction in CI after sunitinib. Because SVRi is calculated as the ratio of MABP and CI, a smaller change in blood pressure can result in a similar change in SVRi. Importantly, no evidence for an increase in sunitinib-induced, ROS-mediated vasoconstriction was found. Of note, activation of NADPH oxidase is also a critical step in the VEGFR signaling cascade. If this pathway is blocked after sunitinib administration, it is possible that activation of NADPH oxidase via the ET-1 pathway is offset by a decreased activation of NADPH oxidase because of VEGFR inhibition. Because VEGF is known to stimulate eNOS via the phosphatidylinositol 3-kinase-Akt pathway through activation of VEGFR-2, the enhanced vasoconstrictor response to acute eNOS inhibition after sunitinib administration observed in the present study was unexpected. This finding implies that the sunitinib-induced rise in MABP is accompanied by an increase in NO-dependent vasodilator tone. Given the complex interaction between NO and ET, it is possible that the enhanced NO-mediated vasodilator tone is attributed to increased ET receptor stimulation while simultaneously limiting ET-induced vasoconstriction. An alternative explanation may be the use of young, healthy swine in the present study, whereas patients with cancer are generally older and may have endothelial dysfunction. By comparing the responses to eNOS inhibition before and after sunitinib in the same swine, we aimed to correct for age and potential underlying vascular disease. Nevertheless, it is possible that the increase in vascular tone in response to sunitinib resulted in an increase in shear stress, thereby enhancing NO production, and that this effect is more pronounced in young healthy swine as compared with patients with underlying cardiovascular disease. Indeed, sunitinib treatment for 8 days was accompanied by a decreased urinary nitrate excretion in adult rats and in patients receiving various inhibitors of the

Table 2. Changes in Hemodynamic Parameters as a Result of Endothelial NO Synthase Inhibition, Endothelin A/B Blockade, and Reactive Oxygen Species Scavenging in the Absence and Presence of Sunitinib

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sunitinib</th>
<th>LNNA</th>
<th>Tezosentan</th>
<th>ROS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔHR, bpm</td>
<td>Control</td>
<td>−11±7</td>
<td>6±3</td>
<td>22±8*</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>−24±3*†</td>
<td>0±5</td>
<td>22±7*</td>
<td></td>
</tr>
<tr>
<td>ΔMABP, mm Hg</td>
<td>Control</td>
<td>24±1*</td>
<td>0±3</td>
<td>−13±2*</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>32±3*†</td>
<td>−12±4*</td>
<td>−5±2*†</td>
<td></td>
</tr>
<tr>
<td>ΔCI, mL·min⁻¹·kg⁻¹</td>
<td>Control</td>
<td>42±8*</td>
<td>12±5</td>
<td>35±6*</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>47±8*</td>
<td>13±4*</td>
<td>22±4*</td>
<td></td>
</tr>
<tr>
<td>ΔLVSP, mm Hg</td>
<td>Control</td>
<td>18±5*</td>
<td>4±3</td>
<td>−9±5</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>24±7*</td>
<td>−9±6</td>
<td>−10±3*</td>
<td></td>
</tr>
<tr>
<td>ΔSvi, mL/kg</td>
<td>Control</td>
<td>−0.23±0.10</td>
<td>0.02±0.04</td>
<td>0.05±0.06</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>−0.16±0.07</td>
<td>0.10±0.03*</td>
<td>−0.03±0.06</td>
<td></td>
</tr>
<tr>
<td>ΔLV dp/dtmax, mm Hg/s</td>
<td>Control</td>
<td>−123±216</td>
<td>−20±123</td>
<td>158±152</td>
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<tr>
<td>Sunitinib</td>
<td>−580±342</td>
<td>217±69*</td>
<td>118±129</td>
<td></td>
</tr>
<tr>
<td>ΔLV dp/dtrelax, mm Hg/s</td>
<td>Control</td>
<td>−185±46*</td>
<td>−27±135</td>
<td>22±78</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>−202±70*</td>
<td>82±170</td>
<td>156±69*</td>
<td></td>
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</table>

HR indicates heart rate; MABP, mean blood pressure; CI, cardiac index; LVSP, left ventricular systolic pressure; Svi, stroke volume index; LV dp/dt, left ventricular rate of change in pressure; LNNA, N⁴-nitro-L-arginine; ROS, reactive oxygen species scavenging.

*P<0.05 effect of LNNA, tezosentan, or ROS scavenging vs corresponding baseline.
†P<0.05 effect of LNNA, tezosentan, or ROS scavenging altered as a result of sunitinib.
VEGF pathway, and the RTKI vandetanib decreases systemic plasma nitrate/nitrite levels in treated patients.\textsuperscript{8,12,38} Results of clinical studies using FMD of the brachial artery as an index of NO bioavailability are conflicting. In one study, administration of the RTKI telatinib\textsuperscript{9} was associated with a decrease in FMD from 6.0% to 3.9%, whereas in another study administration of the RTKI vandetanib had no effect on FMD (12.0% before and 13.8% after vandetanib).\textsuperscript{8} In the study reported by Steeghs et al.,\textsuperscript{9} the vasodilatation to nitroglycerin was also reduced (from 17.0% to 9.7%) to a comparable extent as the decrease in FMD, indicating a diminished response of the vascular smooth muscle cells to NO rather than a selective decrease in NO bioavailability. In the coronary microcirculation of rats exposed to sunitinib for 8 days, we found, using the Langendorff heart model, that the vasodilator responses to both bradykinin (endothelium dependent) and sodium nitroprusside (endothelium independent) were impaired.\textsuperscript{10} Remarkably, in this model we also found an attenuated vasoconstrictor response to angiotensin II. These findings suggest generalized impairment of vascular smooth muscle function during sunitinib administration that is still unexplained. Part of the variable findings between different studies may be explained by the fact that the various RTKIs have different target receptors,\textsuperscript{1} thereby affecting the NO pathway to a greater or lesser extent. Altogether, until now, evidence is lacking to conclude that a decrease in NO bioavailability is a predominant factor in RTKI-induced vasoconstriction.

Our study is the first to investigate potential adverse effects of the RTKI sunitinib on the pulmonary vasculature. Contrary to its vasoconstrictor effect in the systemic circulation, sunitinib did not cause pulmonary vasoconstriction either at rest or during exercise. Variation in expression and function of ET\textsubscript{A} and ET\textsubscript{B} receptors between these circulations may provide an explanation for this difference. The ET\textsubscript{A} receptor predominates in the systemic and coronary vasculature, whereas the ET\textsubscript{B} receptor predominates in the pulmonary microcirculation, where it is present on both the endothelium and the vascular smooth muscle cells and also functions as a clearance receptor.\textsuperscript{39} In a previous study, using the same animal model as in the present study, we found that the dose of ET-1 required to induce vasoconstriction in the systemic circulation is lower than the dose to induce pulmonary vasoconstriction.\textsuperscript{20} Apparently, the sunitinib-induced activation of the ET pathway was not sufficient to induce pulmonary vasoconstriction, thereby precluding adverse effects of RTKI on the pulmonary circulation.

In the present study, administration of sunitinib had no adverse effects on stroke volume or LVdP/dt\textsubscript{max} and LVdP/dt\textsubscript{min}, indices of left ventricular systolic and diastolic function, either at rest or during exercise. Moreover, no evidence was found for coronary vasoconstriction, because CBF did not change during sunitinib administration. In contrast, sunitinib treatment of patients with imatinib-resistant, metastatic gastrointestinal stromal tumors was associated with the development of congestive heart failure in 8% of patients and a decrease in left ventricular ejection fraction of \( \geq 10\%\) in 28% of patients.\textsuperscript{2} This discrepancy might in part be explained by the difference in exposure time to sunitinib, as well as the fact that our intervention was performed in young and healthy animals, whereas in the clinical study\textsuperscript{2} pre-existent coronary artery disease remained as the only statistically significant predictor for congestive heart failure in a multivariate logistic regression model.

**Perspectives**

Angiogenesis inhibition with sunitinib induces a marked rise in MAPB, which is independent of changes in endogenous ROS and NO production but completely reversed by ET receptor antagonism with tezosentan. These findings support previous observations that sunitinib-induced hypertension is ET mediated, whereas decreased NO bioavailability or enhanced oxidative stress does not appear to be involved. Because we showed recently that ET receptor antagonism could also prevent sunitinib-induced proteinuria and urinary ET-1 excretion (markers of renal injury), ET receptor antagonists appear to be logical candidates to counteract the sunitinib-induced cardiovascular and renal adverse effects.\textsuperscript{12} In addition, ET-1 has been shown to promote angiogenesis in cancer, and, therefore, ET-1 receptor antagonism may have complementary therapeutic effects.\textsuperscript{30,41} However, carefully conducted clinical trials are required to demonstrate whether indeed ET receptor antagonists may be the preferred agents to treat the cardiovascular and renal adverse effects associated with antiangiogenesis therapy in patients with cancer.

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**Disclosures**

None.

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