Mechanisms of Hypertension and Renal Injury During Vascular Endothelial Growth Factor Signaling Inhibition

Anton H. van den Meiracker, A.H. Jan Danser

The recognition that angiogenesis is critical for tumor growth has stimulated the development of several agents that interfere with the process of angiogenesis. Of these agents, vascular endothelial growth factor (VEGF) signaling pathway (VSP) inhibitors have gained a prominent role in the treatment of a large variety of cancers. Along with their clinical introduction, treatment with VSP inhibitors appeared to be associated with the development of cardiovascular side effects, particularly hypertension, renal injury, cardiac dysfunction, thrombosis, and hemorrhage. When severe, these side effects are reason to discontinue VSP inhibitor treatment, obviously with repercussions for survival. In this review, we focus on mechanisms underlying the rise in blood pressure (BP) and kidney injury induced by VSP inhibitor treatment. Insight into the mechanisms of these side effects is not only of scientific interest, but may also provide a basis for their prevention with rational treatment. Because for a proportion of clinicians interested in hypertension the VEGF system is not common knowledge, we will start with a short description of this system and ways of its inhibition by pharmacological agents.

VEGF, VEGF Receptors, and VSP Inhibitors

The mammalian VEGF family includes the glycoproteins VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and the placental growth factor, with VEGF-A, commonly referred to as VEGF, as the most prominent factor. The VEGFs are mainly present as homodimeric polypeptides. Alternative splicing and proteolytic processing increase the complexity of the VEGF system. For instance, alternative splicing of the gene encoding VEGF is crucial for the initiation of angiogenesis.8 Different soluble splice variants of the VEGFR-1, consisting of the extracellular part of the VEGFR-1, have been described.9,10 These variants can exert a negative regulatory role by sequestering VEGF-A. In preeclampsia, the circulating concentration of sFlt-1–14a, also known as sFlt-1–14, and originating from placental syncytiotrophoblasts, is massively increased, playing a dominant role in the pathophysiology of this condition.9,11 VEGF is also known to control different processes during neurodevelopment. Of interest with regard to BP homeostasis, transgenic mice with low VEGF levels have defects in the regulation of resistance arteries because of dysfunction of the synapses between autonomic nerve endings and vascular smooth muscle cells and to an impaired vascular smooth muscle cell contractile response.12

VEGF signaling is influenced by several co-receptors.13 Neuropilins 1 and 2 (NRP1 and NRP2) are transmembrane proteins involved in neuronal guidance function. NRP1 is predominantly expressed in monocytes, macrophages, and ECs, VEGFR-2 in vascular ECs, and VEGFR-3 in lymphatic ECs. VEGFR-1 binds VEGF-A, VEGF-B, and PlGF and VEGFR-2 is activated primarily by VEGF-A, whereas VEGFR-3 is activated by VEGF-C and VEGF-D. The affinity of VEGF-A for the VEGFR-1 is ≈10-fold higher than that for VEGFR-2, whereas most of the angiogenic effects of VEGF are mediated via VEGFR-2. VEGFR-1, therefore, mainly functions as a decoy receptor for VEGF, modulating the degree of activation of the VEGFR-2. VEGFR-1 is also expressed in macrophages/monocytes.7,8 VEGF-A has been shown to augment the recruitment of macrophages, promoting lymphangiogenesis as well angiogenesis. Different soluble splice variants of the VEGFR-1, with maintenance of the paracrine VSP leads to progressive EC degeneration and sudden death, indicating requirement of the autocrine VSP for the maintenance of the vitality of ECs.3

The various effects of VEGF are mediated by binding to VEGF receptors (VEGFRs), belonging to the immunoglobulin-like extracellular ligand–binding domains, a transmembrane domain, and a cytoplasmic kinase domain with tyrosine residues. VEGFR-1 or fms-like tyrosine kinase-1 (sFlt-1) is predominantly expressed in monocytes, macrophages, and ECs, VEGFR-2 in vascular ECs, and VEGFR-3 in lymphatic ECs. VEGFR-1 binds VEGF-A, VEGF-B, and PlGF and VEGFR-2 is activated primarily by VEGF-A, whereas VEGFR-3 is activated by VEGF-C and VEGF-D. The affinity of VEGF-A for the VEGFR-1 is ≈10-fold higher than that for VEGFR-2, whereas most of the angiogenic effects of VEGF are mediated via VEGFR-2. VEGFR-1, therefore, mainly functions as a decoy receptor for VEGF, modulating the degree of activation of the VEGFR-2. VEGFR-1 is also expressed in macrophages/monocytes. VEGF-A has been shown to augment the recruitment of macrophages, promoting lymphangiogenesis as well angiogenesis. Different soluble splice variants of the VEGFR-1, consisting of the extracellular part of the VEGFR-1, have been described.9,10 These variants can exert a negative regulatory role by sequestering VEGF-A. In preeclampsia, the circulating concentration of sFlt-1–14a, also known as sFlt-1–14, and originating from placental syncytiotrophoblasts, is massively increased, playing a dominant role in the pathophysiology of this condition. VEGF is also known to control different processes during neurodevelopment. Of interest with regard to BP homeostasis, transgenic mice with low VEGF levels have defects in the regulation of resistance arteries because of dysfunction of the synapses between autonomic nerve endings and vascular smooth muscle cells and to an impaired vascular smooth muscle cell contractile response.

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knockout mice, the development of capillaries and lymphatics is abnormal.15,16

The recognition that tumor-derived VEGF plays a key role in tumor angiogenesis and hence in tumor growth has led to development of a large number of agents, interfering with the VSP in different ways (Table).17 The RTKIs block the phosphorylation of tyrosine residues of the VEGFRs by interacting with their adenosine triphosphate pocket. Related to this action, RTKIs typically target several tyrosine kinases (Table).

Incidence of Hypertension and Renal Injury
Soon after the introduction of VSP inhibitors in the clinic, they appeared to be associated with the development of cardio-renal adverse effects. BP rises in virtually every patient exposed to VSP inhibitor treatment and is thus considered to be a class-specific effect. The development of hypertension and renal injury can vary considerably among clinical trials and clearly is dose-dependent. In a meta-analysis, the incidence of hypertension for a low dose of bevacizumab ranged from 3% to 32% and for a high dose from 18% to 36%.18 For proteinuria, values were, respectively, 21% to 41% and 22% to 63%.18 For ramucirumab, an antibody against VEGFR-2, the incidence of hypertension and proteinuria is relatively low, with reported values of 6% to 16% and 6% to 17%.19 However, dose-limiting hypertension has been observed with this agent in a dose-escalating study, confirming the view that VEGFR-2 blockade is critical for the rise in BP. The incidence of hypertension reported for the different RTKIs shows considerable variation, and especially, high rates of hypertension in over 60% of patients have been reported for the RTKIs cediranib and lenvatinib.20,21 The reported difference in the incidence of hypertension may be explained by the intensity of blockade of the VEGFR-2 induced by the different VSP inhibitors. Furthermore, blockade of additional tyrosine kinases by the different RTKIs may play a role in the development of hypertension either by contributing to or by antagonizing the rise in BP.

Preexisting hypertension is an independent risk factor for BP elevation after VSP inhibition. In addition, age >60 years and body mass index >25 kg/m², but not the different RTKIs applied, emerged as independent risk factors.22 Because the development of hypertension in response to VSP inhibition can occur within hours to days, close monitoring of BP after initiation of a VSP inhibitor is mandatory.23 The development of VSP inhibition–induced hypertension has been reported to associate with prolonged progression-free and total survival in patients with various forms of cancer. However, recent studies could not confirm such an association.24,25

Mechanism of Hypertension
Decrease in NO Bioavailability
Stimulation of the VEGFR-2 activates nitric oxide (NO) production both through upregulation of NO synthase (NOS) gene expression and through increased endothelial NOS phosphorylation.26–28 In mice, inhibition of VEGFR-2 rapidly increases BP and reduces the renal expression of neuronal and endothelial NOS in the kidney, whereas preadministration of an NOS antagonist abolished the rise in BP.29 Based on this knowledge, the potential role of NO deprivation in the development of hypertension during VSP inhibition has been explored in preclinical and clinical studies. In patients with renal cell carcinoma, the urinary excretion of NO metabolites during treatment with bevacizumab was identical to that in controls, but almost halved in patients treated with RTKIs.30 Vandetanib treatment in breast cancer patients was associated with a modest decrease in plasma nitrate/nitrite levels.31 In patients with a gastrointestinal stromal tumor, regorafenib treatment was associated with a reversible suppression of NO.32 In a study in healthy volunteers, intra-arterial infusion of bevacizumab in the forearm acutely reduced the vasodilator response to acetylcholine, consistent with decreased NO availability.33 Conversely, systemic administration of sunitinib for 1 week in patients, only inducing a 5 mmHg BP rise, was not associated with diminished forearm vasodilator response to acetylcholine, leading the authors to conclude that reduced endothelium-dependent vasodilation does not precede the developmental phase of hypertension after sunitinib treatment.34 Interestingly, in this same report, a high dose of sunitinib administered to rats reduced endothelium-dependent vasodilation by lowering NO availability. Our group also observed a decrease in urinary excretion of NO metabolites or its effector molecule cGMP in rats exposed to sunitinib.35,36 In contrast, using the Langendorff-isolated heart model, the vasodilator response to bradykinin and sodium nitroprusside were both diminished as was the vasconstrictor response to angiotensin II.37 These findings suggest a general
disturbance in vascular smooth muscle cell function rather than a decrease in NO availability. In a study performed in awake swine, the rise in BP induced by the NOS inhibitor N-nitro-l-arginine was increased after 1 week administration of sunitinib, causing a 12 mmHg rise in mean arterial pressure, implying an increase rather than a decrease in NO availability. Collectively, although not uniformly positive, the studies reviewed here support the view that VSP inhibition associates with a decrease in NO availability, contributing to the rise in BP.

**Activation of the Endothelin System**

In a study in patients with metastatic renal cell and gastrointestinal stromal cancer aimed to obtain insight into a potential role of neurohormones in the rise in BP associated with VSP inhibition, we were the first to observe that the BP rise induced by sunitinib was accompanied by a 2-fold increase in circulating endothelin-1 (ET-1) levels. Plasma renin concentration in this same population decreased, whereas plasma catecholamines did not change. Studies in rats exposed to different oral doses of sunitinib confirmed the dose-dependent activation of the endothelin system after VSP inhibition. Activation of the endothelin system has also been observed with the RTKI regorafenib as well as in preeclampsia. In preeclampsia, the rise in circulating ET-1 levels is directly related to the rise in the endogenous VEGF inhibitor sFlt-1. As demonstrated in mice, showing a 65% to 85% decrease in ET-1 levels after knockout of the ET-1 gene in ECs, the main source of circulating ET-1 levels is vascular ECs. Whether the observed rise in ET-1 after VSP inhibition is directly related to VEGF deprivation in ECs or indirectly to activation of these cells or both remains uncertain because of conflicting experimental findings. Adding VEGF to human umbilical ECs increases ET-1 gene expression and ET-1 protein production. In contrast, in human bronchial microvascular ECs, VEGF dose-dependently decreased ET-1 excretion. Importantly, the RTKI SU5416 could abolish this decreased ET-1 excretion.

Additionally to increased ET-1 levels, overexpression of sFlt-1 in mice is associated with increased renal mRNA expression of preproET-1 and the endothelin type A (ET<sub>A</sub>) receptor, an effect further enhanced in eNOS<sup>−/−</sup> mice. Furthermore, in mice exposed to sFlt-1, the vasoconstrictor response to ET-1 is increased. Collectively, these findings indicate that increased activity of the ET-1 system after VSP inhibition may be mediated by multiple pathways, including diminished NO availability.

Experimental studies with endothelin receptor antagonists (ERAs) have provided evidence that the rise in ET-1 is instrumental for the rise in BP induced by VSP inhibition. In rats, the rise in BP induced by sunitinib could be prevented almost completely by the ET<sub>A</sub> ERA macitentan, whereas in this same model, captopril and sildenafil were devoid of any BP-lowering effect. Also, in instrumented awake swine, the sunitinib-induced rise in BP could be completely reversed to pre-sunitinib values by the ERA tezosentan.

**Rarefaction and Oxidative Stress as Other Potential Hypertensive Mechanisms**

Loss of ECs within tumors is observed after initiation of VSP inhibition in cancer xenograft models. Not surprisingly, therefore, rarefaction has been a proposed mechanism involved in the development of hypertension after VSP inhibition. Clinical evidence for a small decrease in capillary density has been obtained during treatment with bevacizumab, telatinib, and sunitinib, but rarefaction in our view plays at best a marginal role in VSP inhibition–induced hypertension. First, the rise in BP after initiation of antiangiogenic treatment occurs fast, whereas discontinuation of this treatment is associated with a rapid BP normalization. Second, a mathematical model based on the hamster cheek pouch microcirculation indicates that for a 5% increase in vascular resistance, 42% rarefaction of fourth order arterioles is required. That such an extensive degree of rarefaction ever occurs during VSP inhibition is unlikely. Finally, in swine, we observed that the decrease in systemic and coronary vascular resistance in response to treadmill exercise was identical before and after sunitinib administration.

Enhanced oxidative stress may be another mechanism contributing to the development of hypertension in response to VSP inhibition. Activation of the endothelin system during VSP inhibition may also result in oxidative stress through activation of the ET<sub>A</sub> receptor/NADPH oxidase pathway. Our group has explored the possibility whether an increase in oxidative stress occurs during VSP inhibition and whether it is involved in the development of hypertension. In rats exposed to sunitinib, inducing a 30-mmHg BP rise, we observed no increase in urinary excretion of thiobarbituric acid reactive substances, and the BP rise was hardly diminished by coadministration of tempol. Likewise, in swine, the rise in BP induced by sunitinib could not be lowered by a cocktail of antioxidants, whereas the same cocktail lowered BP in sunitinib-naive animals. Collectively, these observations do not support the idea that increased oxidative stress contributes to the development of hypertension induced by VSP inhibitors.

**Salt-Sensitivity of BP**

Two studies have explored whether the rise in BP induced by VSP inhibition is salt-sensitive. In normotensive Sprague–Dawley rats, the RTKI SU5416 induced salt-sensitive hypertension and kidney injury. In a study performed in normotensive Wistar–Kyoto rats exposed to a low dose of sunitinib, we also found that a high salt diet augmented the rise in BP and proteinuria, whereas kidney excretory function was unaffected. Different mechanisms may account for the salt-sensitive hypertension during VSP inhibition. As suggested by Gu et al, the VSP inhibition–induced decrease in NO production by renal proximal tubular cells may impair the pressure-natriuresis response because of impaired vasodilatation in the vasa recta. In addition, the observed VSP inhibition–associated renin suppression and activation of the endothelin system may contribute to salt-sensitive hypertension. In response to a high salt diet, sodium and chloride accumulate in the skin in excess of water, leading to a hypertonic interstitial fluid compartment. In response to this hypertonicity,
Figure. Scheme showing mechanism of hypertension after vascular endothelial growth factor (VEGF) signaling pathway (VSP) inhibition. Interrupted line: mechanism not yet established. ET \textsubscript{A} indicates endothelin type A.

dermal mononuclear phagocyte system cells produce increased amounts of the tonicity-responsive enhancer-binding protein, a transcription factor that initiates expression of VEGF-C. VEGF-C, in turn, stimulates the formation of lymph vessels as an adaptive mechanism to clear the excessive electrolytes. Interruption of this pathway in mice and rats associates with salt-sensitive hypertension. Whether this mechanism also contributes to the salt-sensitive hypertension during VSP inhibition remains to be clarified.

**Mechanism of Kidney Injury**

**Direct Effect of VEGF Deprivation**

VEGF signaling plays a fundamental role in the development and maintenance of kidney structure and function. VEGF is highly expressed in podocytes and interacts in a paracrine way with glomerular ECs through activation of VEGFR-2. Administration of anti-VEGF antibodies to mice or injection of adenovirus encoding for sFlt-1 in pregnant and nonpregnant rats is associated with development of proteinuria. Selective depletion of one VEGF allele in podocytes in mice leads to downregulation of the slit-diaphragm protein nephrin, resulting in proteinuria and glomerular endotheliosis. Similar renal abnormalities have been observed in animals treated with VSP inhibitors and in patients with preeclampsia. Recently, selective embryonic excision of the gene encoding for VEGF-A from renal tubular cells has been shown to result in the formation of a smaller kidney with a striking reduction in peritubular capillaries and polycytemia. In rats exposed to the RTKI sunitinib, no evidence for a decrease in peritubular ECs was found, and also in renal biopsies obtained from patients treated with VSP inhibitors, glomerular abnormalities are predominant. In addition to the mentioned podocyte-glomerular and tubulo-vascular cross talk between VEGF and VEGFR-2, recent evidence indicates an autocrine role of the soluble part of the VEGFR-1 produced by podocytes to maintain the glomerular filtration barrier. Podocyte-specific deletion of the gene encoding VEGFR-1 results in disruption of the glomerular cytoskeletal architecture and heavy proteinuria. The development of this phenotype is independent of the tyrosine kinase domain of the Flt-1 receptor.

**Role of Activation of the Endothelin System**

As described, VSP inhibition is associated with an activated endothelin system, consistent with an activated state of ECs because of deprivation of VEGF activity. Also the urinary excretion of ET-1, reflecting increased renal ET-1 production, is enhanced after VSP inhibition. One may pose the question, therefore, whether activation of the ET system contributes to the renal injury induced by VSP inhibition. Using human cultured podocytes, Collino et al have shown that conditioned medium obtained from glomerular epithelial cells incubated with sera from patients with PE induced shedding of nephrin and synaptopodin, 2 structural proteins of the podocyte slit-diaphragm. Nephrin shedding could be abrogated with an ERA. A chronic subpressor dose of ET-1 in normotensive rats is associated with increased glomerular permeability to albumin and an increase in nephrin excretion rate, an effect that can be inhibited by an ET \textsubscript{A} receptor antagonist. Using cultured mouse podocytes, it has recently been shown that ET-1 promotes podocyte migration via ET \textsubscript{A} receptor activation and increased β-arrestin-1 expression. We found in rats that the proteinuria induced by sunitinib could be inhibited by the dual ERA macitentan. This anti-proteinuric effect of macitentan was likely BP-independent because an identical BP-lowering effect induced by amiodipine was associated with an increase in proteinuria.

**Histopathology of Kidney Biopsies of Patients Treated With VSP Inhibitors**

Thrombotic microangiopathy (TMA) is a well-established complication of VSP inhibitor treatment. However, a recent study indicates a more diverse spectrum of renal histological abnormalities after VSP inhibition. Kidney biopsies of 100 patients with proteinuria and renal function impairment during VSP inhibition showed TMA in 73 and minimal change and collapsing-like focal segmental glomerulosclerosis in 27 patients. In 50% of patients with TMA, no systemic hematologic manifestations of TMA were detectable. Almost all patients with TMA were treated with bevacizumab or VEGF-trap, whereas all but one of the patients with minimal change and collapsing-like focal segmental glomerulosclerosis were treated with a RTKI. Age, proteinuria, and renal function impairment did not differ, but BP was higher in the TMA group. Patients with TMA displayed a high abundance of RelA, a nuclear factor NF-kappa subunit, in podocyte nuclei and ECs, but c-maf-inducing protein was not detectable. In contrast, patients with minimal change and collapsing-like focal segmental glomerulosclerosis –like lesions showed high abundance of c-maf-inducing protein expression in podocytes, whereas RelA was hardly detectable. These findings suggest that the renal histopathology induced by VSP inhibition is less uniform than originally thought because of different pathological mechanisms in part depending on the way VSP inhibition is accomplished.
Considerations About Antihypertensive Treatment

In 2012, the Cardiovascular Toxicities Panel of the National Cancer Institute has launched recommendations about the management of cardiovascular toxicity in patients receiving VSP inhibitors. The panel recommends (1) a formal cardiovascular risk assessment before initiation of VSP inhibitor treatment, (2) active monitoring of BP and cardiac toxicity throughout treatment, with more frequent monitoring during the first cycles of therapy, given that marked and unpredictable BP rises can occur early after treatment with a VSP inhibitor, and (3) aggressive management of BP elevations and early symptoms and signs of cardiac toxicity to prevent clinically limiting complications. In patients with preexisting hypertension, the BP target for initiating VSP inhibitor treatment should be <140/90 mm Hg.

Because clinical trials about the most optimal treatment of hypertension or renal injury induced by VSP inhibitors are not available and unlikely are ever to be performed, advice about treatment of these adverse effects can only be based on insight into pathophysiological mechanisms and data obtained in preclinical studies. In rats exposed to sunitinib, resulting in a 30 mm Hg rise in mean arterial pressure, heavy proteinuria, and impaired renal excretory function, we have tested the BP-lowering and antiproteinuric effect of different antihypertensive agents. We observed that the calcium channel blocker amlopidine and the dual ERA macitentan could prevent the rise in BP. In contrast, the angiotensin-converting enzyme inhibitor captopril and the phosphodiesterase inhibitor sildenafil reduced proteinuria, but these agents had no effect on BP. Interestingly, macitentan also reduced proteinuria. These experimental findings agree well with previous clinical and experimental studies that the hypertension induced by VSP inhibition is a low-renin, high-ET-1, salt-sensitive form of hypertension. Based on these data, we propose to use a dihydropyridine calcium channel blocker as a first line treatment of hypertension. In case of proteinuria, an angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker should be added. An ERA may be a valuable alternative, but such agents are not approved for the indication of hypertension or renal injury. The use of ERAs may also be hampered by potential pharmacokinetic interaction because many ERAs as well as RTKs are metabolized by CYP3A4. Furthermore, currently available ERAs are expensive.

Conclusions

The introduction of VSP inhibitor treatment has unmasked a role of the baseline activity of the VEGF system for BP regulation and maintenance of renal function. The hypertension and renal injury induced by VSP inhibition resemble those of preeclampsia and share an identical pathophysiological mechanism (Figure). Evidence indicates that activation of the endothelin system, most likely as a reflection of an activated state of ECs because of VEGF deprivation, is a mediator of the rise in BP and, to some extent, also of renal injury. The hypertension induced by VSP inhibition in most cases is well treatable, but the development of renal injury, especially TMA, is mostly a reason to discontinue VSP inhibitor treatment.

References


Disclosures

None.


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