Molecular basis of hypertension side effects induced by sunitinib

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Over the past decade a number of vascular complications have emerged, such as newly developed or worsened hypertension, in patients who were administered with new cancer treatments for several types of cancer that were untreatable earlier. Hypertension is emerging as one of the most common adverse effects of therapy with angiogenesis inhibitors. Small-molecule inhibitors of vascular endothelial growth factor signalling are associated with a high proportion of patients with hypertension. The mechanisms underlying the development of hypertension are not well known, although there seem to be several mechanisms. Physiopathology of hypertension implicates abnormalities in endothelial function and angiogenesis. Several features of hypertensive patients are reduced number of arterioles and capillaries, alterations of the microvascular network, decrease in vascular wall compliance and flexibility, reduced nitric oxide bioactivity and increases in plasma vascular endothelial growth factor. Treatment with tyrosine kinase inhibitors (TKIs) is associated with a significant and sustained increase in blood pressure. We suspect that TKIs exert their hypertensive effects directly at the level of the microvascular network through processes such as vascular rarefaction, endothelial dysfunction and/or altered nitric oxide metabolism. This study shows the vascular complications of treatment with a TKI, sunitinib (SU11248), with special emphasis on hypertension. ***Anti-Cancer Drugs*** **22:1–8 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Sunitinib is a multitargeted tyrosine kinase inhibitor (TKI) used in the treatment of metastatic renal cell carcinomas (RCC) and gastrointestinal stromal tumours, and is under evaluation for other malignancies.

Hypertension is one of their major side effects with a substantial variation in the reported incidences among clinical studies. Among patients receiving sunitinib, the incidence of all-grade and high-grade hypertension was 21.6 and 6.8%, respectively, which was associated with a significantly increased risk of high-grade hypertension [1].

Sunitinib malate (Sutent; Pfizer, New York, USA) is a small-molecule inhibitor of certain receptor tyrosine kinases, including the vascular endothelial growth factor receptor (VEGFR) type 1 and type 2 (FLT1 and FLT1/KDR), platelet-derived growth factor receptors (PDGF-α, PDGF-β), stem cell factor receptor (c-KIT) and FLT3 and RET kinases.

Angiogenesis inhibitors act, on the one hand, by blocking the extracellular binding of vascular endothelial growth factor (VEGF) to its receptor by anti-VEGF antibodies, and on the other hand, by blocking the intracellular signalling pathway of VEGFR, inhibiting the signal transmission by receptor tyrosine kinases.

Both VEGFR-2 and PDGFR-β are the major expression subtypes of the VEGFR/PDGFR system in the capillary vasculature. It has been hypothesized that sunitinib acts by inhibiting the effects of VEGFR and PDGFR that cause damage to the capillary endothelium [2]. In fact, recent studies [3] suggest that downregulation or neutralization of circulating VEGF may play an important role in the induction of hypertension.

Hypertension was one of the most common adverse events reported in phase III studies compared with the other therapeutic agents used in RCC treatment [4].

Hypertension has been recorded according to versions of the Common Terminology Criteria for Adverse Events of National Cancer Institute [5,6] (Table 1). In general, normal blood pressure for adults consists of a systolic blood pressure reading of less than 120 mmHg and a diastolic blood pressure reading less than 80 or 120/80 mmHg.

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Table 1 Hypertension grading

NCI (5)		JNC7 equivalence [6]
Grade	Hypertensive adverse event and recommendations	Class
0	None	Normal SBP Prehypertension
1	Asymptomatic, transient (<24 h) increase by >20 mmHg (diastolic) or to >150/100 mmHg if within normal limits earlier; not requiring treatment	Normal Prehypertension
2	Recurrent, persistent or symptomatic increase by >20 mmHg (diastolic) or to >150/100 mmHg if within normal limits earlier; monotherapy may be indicated	Stage 1 hypertension Stage 2 hypertension
3	Requiring more than one drug or more intensive therapy than earlier	
4	Life-threatening consequences (hypertensive crisis)	

NCI, National Cancer Institute; SBP, systolic blood pressure.

The prevalence of hypertension in the cancer-affected population, before the administration of TKI agents, has been estimated to be similar to that of the general population [7,8].

Clinical findings

Hypertension is emerging as one of the most common adverse effects of therapy with angiogenesis inhibitors such as sunitinib.

Among patients receiving sunitinib, the incidence of all-grade and high-grade hypertensions were 21.6% [95% confidence interval (CI): 18.7–24.8%] and 6.8%, respectively (95% CI: 5.5–8.8%). With regard to the tumor type and dosing schedule, sunitinib was associated with a significantly increased risk of high-grade hypertension (RR = *22.72*, 95% CI: 4.48–115.29, $P < 0.001$) [1]. Data collected from the phase II and phase III clinical trials showed the incidence of hypertension ranging from 15% (4% of the patients had grade 3) to 28% (6% of the patients had grade 3) [9–11] with a relative risk of 3.9 using sunitinib [12]. The appearance of hypertension, particularly grade 3, was associated with a higher response to treatment with sunitinib in metastatic RCC [13]. Drug-related hypertension can occur with drug initiation (during the second cycle) [14] and within the first year of treatment. Hypertension symptoms begin with a 20 and 10% increase in the systolic and diastolic blood pressure, respectively.

The incidence and severity of hypertension in cancer patients are dependent on the type of drugs (i.e. sunitinib, sorafenib, bevacizumab) and dosing schedule used, age of patients, and the presence of coexistent cardiac diseases. Poorly controlled hypertension could lead to serious cardiovascular events. In addition, the use of sunitinib may be associated with reversible posterior leukoencephalopathy syndrome a significant event likely to occur secondary to hypertension [2,15].

Mechanisms of hypertension

Mechanisms that induce primary or essential hypertension remain unknown and it is possible that the term encompasses a group of disorders with diverse etiologies. Although the basic determinants of blood pressure are cardiac output and peripheral vascular resistance, the biomolecular cause or causes of hypertension presumably lie in the myriad factors that control these values. In the 1990s, a hypothesis about the abnormalities of both vascular structure and function as the cause of increased vascular resistance was proposed. Measured blood pressure is the product of cardiac output by systemic vascular resistance (SVR). Drugs that increase either one of these are known to increase blood pressure. Thus, hypertension induced by antiangiogenic drugs is probably related to an increase in SVR.

Mechanisms inducing SVR include neurohormonal factors, vascular rarefaction (decrease in the density of microvessels) and endothelial dysfunction associated with a decrease in nitric oxide (NO) production and an increase in oxidative stress.

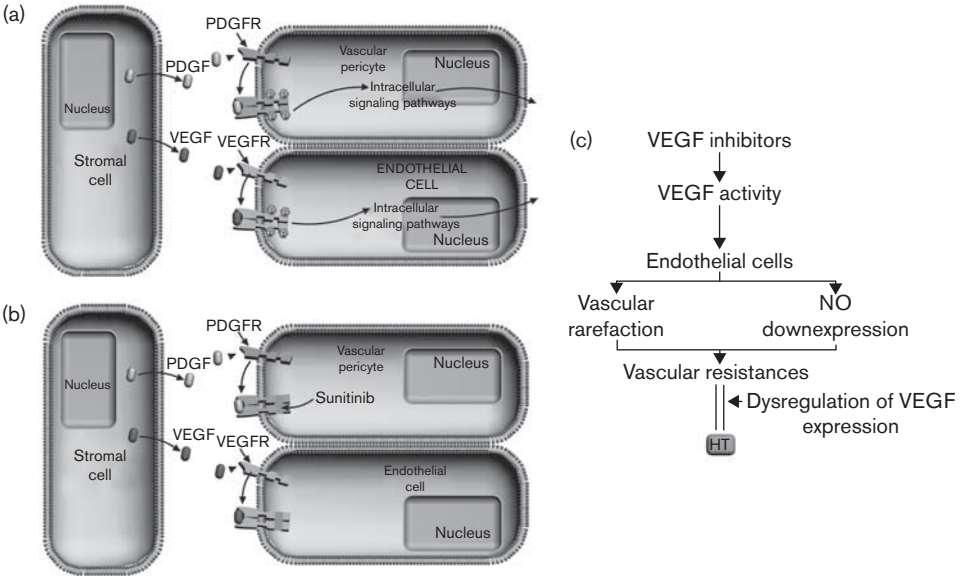
A considerable amount of evidence suggests a link between hypertension and impaired angiogenesis. Several features of hypertensive patients, including the reduced number of arterioles and capillaries, alterations in the microvascular network, decrease in the compliance and flexibility of the vascular wall, reduced NO bioactivity, and increases in plasma VEGF are associated with angiogenesis and its control. Specifically, some investigators have suggested that hypertension results from depressed angiogenesis at the microcirculation level, as is reflected by the phenomenon of rarefaction, which is a reduction in the density of microvessels [16–20]. The resultant diminution of the vascular surface areas leads to increased peripheral vascular resistance (Fig. 1).

However, it is now evident that vascular tone is controlled not only by nervous and hormonal influences but also by the locally active factors produced by the endothelium.

As there were not many significant changes in the humoral factors in the TKI-treated patients experiencing hypertension [21], impaired angiogenesis or endothelial dysfunction may be the cornerstone mechanism of elevated blood pressure.

Arguably, the most important of these mechanisms is NO, whose role as an active vasodilator is now firmly established [22,23]. It is interesting to note that high levels of VEGF have been reported in hypertensive patients [24], and other studies have shown that VEGF exerts its angiogenic effects by enhancing the transcriptional activity of endothelial NO synthase (eNOS) [25]. This observation suggests that VEGF could rapidly induce a hypotensive response. VEGF increases NO synthesis through the upregulation of eNOS, and VEGF inhibition diminishes NO synthesis [24,26]. An important part of the mechanism of hypertension associated with VEGF

Fig. 1



(a) Signal interchange between stromal cells and vascular wall cells (pericytes and endothelial cells). Stromal cells release a number of angiogenic cytokines among which are vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) that bind to specific tyrosine kinase receptors [VEGF receptor (VEGFR) and PDGF receptor (PDGFR)] located on the surface of vascular wall cells. Thus, signal flows into the vascular wall cells to trigger adequate responses such as proliferation, neoangiogenesis and neovasculogenesis. (b) Sunitinib is a small molecule that blocks multiple tyrosine kinase receptors (thus, it is grouped into tyrosine kinase inhibitors). Sunitinib is able to block the sending of signals from VEGFR and PDGFR towards the vascular wall cell, and can therefore inhibit the corresponding specific responses (proliferation, angiogenesis and vasculogenesis). The inhibition of these processes may be a key factor in the development of hypertension. (c) Another mechanism by which hypertension may be achieved is through VEGF inhibition (e.g. by specific antibodies), which may also lead to vascular rarefaction and inhibition of nitric oxide (NO) generation. Thus, it may be triggered vascular resistances and ultimately hypertension.

inhibition is thought to involve decreased production of NO in the wall of arterioles and other resistance vessels. Indeed, VEGF inhibition may cause increased SVR [27,28] and vascular rarefaction [29] leading to hypertension.

Earlier studies suggest that the downregulation or neutralization of circulating VEGF may play an important role in the induction of hypertension [4], and also that hypertension has been shown to be induced by RAF kinase inhibition [30,31].

Nitric oxide pathway

NO is produced by the enzyme NOS during the oxidation of the amino acid substrate L-arginine to L-citrulline [32]. Three distinct NOS isoforms are found in mammalian cells: eNOS (NOS III), neuronal NOS (nNOS or NOS I) and inducible NOS (iNOS or NOS II) (Table 2).

Under normal physiological conditions, iNOS is undetectable in tissues, and thus seems to play little, if any, role in the normal functioning of the cardiovascular system. Both eNOS and nNOS are constitutively expressed, and are thought to contribute to the normal regulation of the vasomotor tone of blood pressure (Fig. 2). There are two principal pathways that control vasodilation: the classical endothelial-dependent NO pathway and the additional central neural-dependent NO pathway.

Table 2 Nitric oxide synthase isoenzymes

Old nomenclature	New nomenclature	Expression
Neuronal NOS	NOS I	Constitutive
Inducible NOS	NOS II	Inducible
Endothelial NOS	NOS III	Constitutive

NOS, nitric oxide synthase; eNOS, endothelial NOS; iNOS, inducible NOS; nNOS, neuronal NOS.

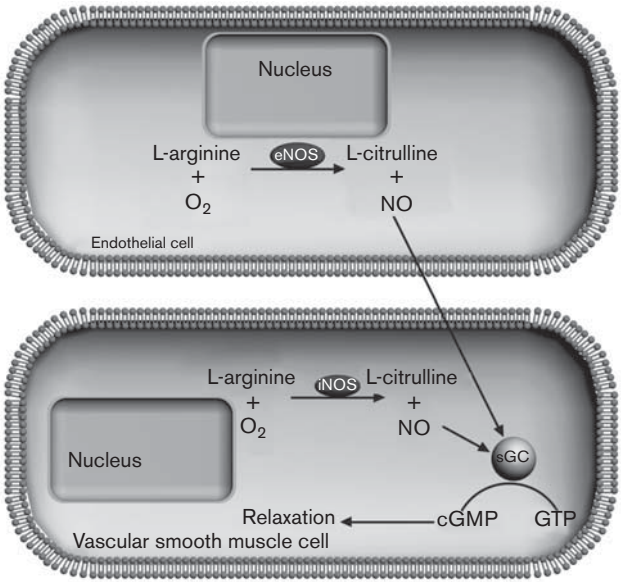
In the vascular endothelium, eNOS converts L-arginine to NO, which diffuses into the adjacent vascular smooth muscle where it activates a series of G-kinases culminating in vasodilation [33] (Fig. 3). In addition, both nNOS and eNOS are expressed in the central nervous system as important components of the signal transcription pathway [34].

Thus, NO is thought to reduce the peripheral vasomotor tone and blood pressure, both by causing active vasodilation and increasing the central sympathetic vasoconstriction drive.

Nitric oxide synthase expression and hypertension

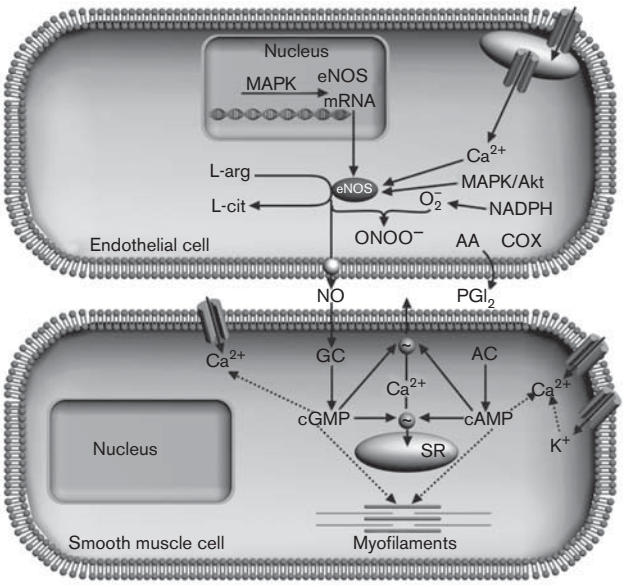
NO, an endogenous vasodilator, synthesized in the endothelium by constitutive NOS inhibits growth-related responses to injury in vascular cells. The endothelium plays a crucial role in the regulation of vascular tone and vascular remodelling [35,36]. NO synthesized by constitutive

Fig. 2



Endothelial cells are responsible for nitric oxide (NO) production. This generation is carried out by endothelial nitric oxide synthase (eNOS) (upper image). Next, NO is released and diffuses into vascular smooth muscle cells. In addition, NO may also be produced in vascular muscle cells through the action of inducible nitric oxide synthase (iNOS). NO is able to enhance the activity of soluble guanilate cyclise (sGC), which transforms GTP to cyclic guanosine monophosphate (cGMP), a second messenger that triggers a number of cell responses to give rise to vascular muscle relaxation (lower image).

Fig. 3



Signalling pathways acting on nitric oxide (NO) generation. In endothelial cells, the mitogen-activated protein kinase (MAPK) signal triggers the activation of endothelial nitric oxide synthase (eNOS) expression. In addition, the MAPK signal together with the Akt signal and Ca^{2+} may enhance eNOS activity and, therefore, NO production. NO is released into the vascular smooth muscle cell where it is able to trigger a relaxation response in myofilaments. cGMP, cyclic guanosine monophosphate; GC, guanylate cyclase.

eNOS is an endogenous vasodilator, which inhibits vascular smooth muscle and mesangial cell growth and, therefore, may participate in vascular and glomerular remodelling in response to hypertensive injury [36,37].

The L-arginine-NO pathway plays an important role in hypertension, both by production of NO and also by interacting with the renin–angiotensin system, eicosanoid pathway and endothelium. It has been reported that NO production was reduced in patients with essential hypertension than in normotensive individuals. In addition, it has been shown that brief elevations induced pharmacologically in blood pressure result in an increased release of NO to the circulation, which can be detected by measuring a small variation in plasma nitrate [38]. In contrast, a fall in systemic pressure results in a decreased production of NO.

In-vitro studies have shown that hemodynamic forces, such as shear stress [39] and cyclin strain, [40] increase vascular NO production by increasing eNOS expression, NOS protein and NOS activity.

NOS activity is a consequence and not a cause of hypertension. Furthermore, clinical studies in humans have suggested that impaired endothelium-dependent relaxations mediated by NO may not be a universal finding in hypertension [41].

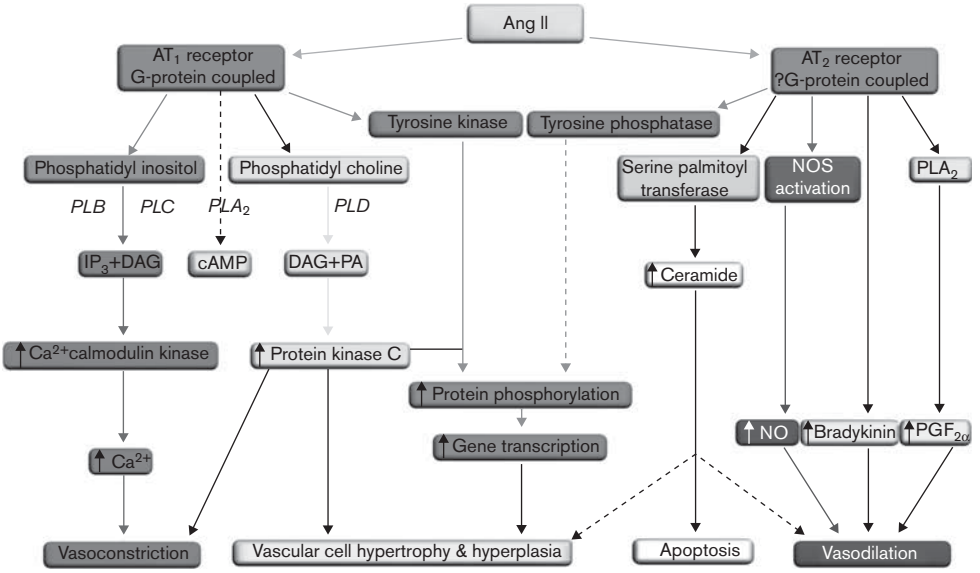
The effects of NO are antagonistic to the effect of angiotensin II (Ang II). The association between the increased activity of the local tissue renin–angiotensin system and vascular pathophysiology has been well documented [36]. NO seems to be the major endogenous antagonist of the vascular actions of Ang II and, therefore, a balance between Ang II and NO seems pivotal for the maintenance of vascular homeostasis [36] (Fig. 4).

Angiotensin-converting enzyme (ACE) plays a major role in the regulation of vascular tone by converting the biologically inactive decapeptide angiotensin I into the vasoconstrictor octapeptide Ang II. Several studies have reported the regulation of ACE expression/activity by NO. Thus, chronic inhibition of eNOS led to the up-regulation of vascular ACE activity [42]. An inverse relationship between ACE expression/activity and the NO system was found in hypertensive rats after a long-term ACE inhibition [43].

Endothelial nitric oxide synthase and hypertension

NO, generated from its precursor L-arginine by NOS, triggers its principal biological actions including vascular smooth muscle relaxation through soluble guanylate cyclase and production of the second messenger cyclic guanosine monophosphate (cGMP) (Fig. 4) NO produced by the vascular endothelial cells diffuses into the

Fig. 4



Multiple roles for angiotensin II (Ang II). Ang II, through its two receptors, is able to trigger a number of responses on the vascular wall cells. These responses are vasoconstriction, vasodilation, apoptosis, vascular cell hyperplasia and hypertrophy. Ang II may trigger through its AT_2 receptor nitric oxide synthase (NOS) activation and nitric oxide (NO) generation. Thus, Ang II is able to produce vascular relaxation and vasodilation. Ang II, through its two receptors, may also act on tyrosine kinases and thyrosine phosphatases to give rise to uncontrolled proliferation and vascular cell hyperplasia and hypertrophy. Finally, Ang II may act through its AT_1 receptor to lead to vasoconstriction, an action that is mediated by phosphatidyl inositol and calcium signalling.

smooth muscle layer where it stimulates the enzyme soluble guanylate cyclase to produce cGMP and causes vasorelaxation. A number of studies have examined both basal and stimulated vascular NO release in human hypertensive populations: basal production of vascular NO has been shown to be impaired in human hypertensive individuals [44,45]; stimulated vascular NO release (endothelial function) has also been shown to be impaired [46–48]. However, not all human trials have confirmed these abnormalities of vascular NO bioactivity in hypertension [49,50].

Neuronal nitric oxide synthase and hypertension

nNOS has been observed in discrete neuronal populations localized within the central nuclei and peripheral autonomic pathways concerned with the regulation of cardiovascular activity [51,52]. Several studies [53,54] have found that nNOS is present in a specific area of the brain involved in the neural control of blood pressure. The neural isoform of nNOS is involved in 10 transduction pathways that inhibit the sympathetic outflow from the brainstem [55].

Nitric oxide synthase inhibitors as a cause of hypertension

The clearest evidence that NO is important in blood pressure regulation comes from the studies that manipulate the L-arginine/NO pathways. Although not to be rate limiting for the eNOS enzyme, the administration of exogenous L-arginine has been shown to reduce blood pressure in normotensive and hypertensive individuals [56]. Chronic inhibition of NOS with L-arginine analogues, such as NG-nitro-L-arginine methylester, produces a dose-dependent and time-dependent, deep, and sustained increase in blood pressure [57]. In humans, blocking NOS in normal individuals with an alternative L-arginine analogue, monomethyl-L-arginine, produced a 10% increase in the mean arterial pressure [58].

Asymmetric dimethyl arginine is an endogenous competitive inhibitor of NOS [59]. It inhibits vascular NO production to concentrations found in pathophysiological conditions.

Nitrovasodilators such as glyceryl trinitrate, isosorbide mononitrate and sodium nitroprusside act by releasing NO into the vascular smooth muscle. They donate NO through mechanisms involving the thiol groups of intracellular proteins [60].

Sodium nitroprusside produces hypotension by increasing the NO-mediated generation of cGMP, which in turn causes vasodilation. In addition, phosphodiesterase type 5 inhibitor augments nitroprusside-induced hypotension [61] (Table 3).

Hypertension management

Patients receiving sunitinib should be monitored for hypertension, ideally at the patient's home, particularly during the first 6 weeks of treatment and in those with preexisting hypertension [62]. A suitable control

Table 3 Nitric oxide production inhibitors

Endogenous NO inhibitors
Methylated arginines (putative endogenous NOS inhibitors)
Asymmetric dimethylarginine
Symmetric dimethylarginine
Exogenous NO inhibitors
Statin drugs
HMG-CoA reductase inhibitors
Estrogen replacement
Angiotensin-converting enzyme inhibitors
Nitric oxide donors
Nitrates (nitroglycerin)
Phosphodiesterase inhibitors (sildenafil)

HMG-CoA, hydroxymethylglutaryl-CoA; NO, nitric oxide; NOS, NO synthase.

of arterial tension must be achieved before beginning treatment with sunitinib. Standard antihypertensive medication should be started as soon as is necessary in patients whose blood pressure increases.

Despite the fact that almost all the available classes of antihypertensive drugs can be used, it is clear that not all antihypertensive agents are equally effective in preventing the progression of hypertension-related chronic renal failure, particularly in the patients who have undergone nephrectomy. Lowering of blood pressure by intervention in the rennin–angiotensin–aldosterone system has an additive renoprotective effect. Both the ACE inhibitors and the Ang II receptor blockers are able to lower the intraglomerular pressure, independent of any change in the systemic blood pressure, by dilation of the efferent arteriole of the glomerulus. Nondihydropyridine calcium channels blockers, such verapamil and diltiazem, should be avoided because they are known CYP3A4 inhibitors [63,64]. However, as amlodipine has minor 3A4 metabolism, it can be used if the blood pressure is difficult to control [62]. Other antihypertensive drugs may also potentially interact with cytocrome P450 and sunitinib. Some of these drugs are the ACE inhibitors, captopril and enalapril, the α-blocker and β-blocker labetalol, the selective β-blocker metoprolol and the Ang II receptor blockers losartan, candesartan and irbesartan [65].

Patients diagnosed earlier with hypertension may require adjustment of the antihypertensive medication during sunitinib therapy. An example of the possible treatment protocol in patients with hypertension during the therapy could begin with hydrochlorothiazide: 12.5 mg qd [quot dian (every day)] and increase until the maximum/tolerable dose. If hypertension is not controlled, then add another drug such as atenolol (25–100 mg qd), telmisartan (40–80 mg qd) or valsartan (80–160 mg qd). If blood pressure is not controlled with these antihypertensive drugs, amlodipine (2.5–10 mg qd) may be used. If therapy for hypertension is required only during the treatment phase, it may be discontinued when the patients have completed the treatment with sunitinib. The objective of antihypertensive treatment is to normalize blood pressure (resting rate $< 140/90$ mmHg). Temporary suspension of sunitinib is recommended by some investigators for patients with severe hypertension (> 200 mmHg systolic or > 110 mmHg diastolic) and resumed once hypertension is controlled [64].

Caution should be taken when sunitinib is used in combination with other drugs that also cause QT and PR interval prolongation [62].

Careful attention must be paid to patients treated with diuretics because of the high risk of diarrhoea and dehydration (up to 60% of patients treated with sunitinib develop diarrhoea). Dihydropyridine must be used with caution in patients on sunitinib treatment (risk of causing peripheral edema) as dihydropyridine predominantly dilates the precapillary limb in the vascular tree, increasing the transcapillary pressure gradient and forcing fluid out of the vascular compartment into the tissue, resulting in oedema. [66–68].

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