

REVIEW

## **Pathophysiological mechanisms of calcineurin inhibitor-induced nephrotoxicity and arterial hypertension**

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## Summary

Solid organ transplantation is an established treatment modality in patients with end-stage organ damage in cases where other therapeutic options fail. The long-term outcomes of solid organ transplant recipients have improved considerably since the introduction of the first calcineurin inhibitor (CNI) - cyclosporine. In 1984, the potent immunosuppressive properties of another CNI, tacrolimus, were discovered. The immunosuppressive effects of CNIs result from the inhibition of interleukin-2 synthesis and reduced proliferation of T-cells due to calcineurin blockade. The considerable side effects that are associated with CNIs therapy include arterial hypertension and nephrotoxicity. The focus of this article was to review the available literature on the pathophysiological mechanisms of CNIs that induce chronic nephrotoxicity and arterial hypertension. CNIs lead to activation of the major vasoconstriction systems, such as the renin-angiotensin and endothelin systems, and increase sympathetic nerve activity. On the other hand, CNIs are known to inhibit NO synthesis and NO-mediated vasodilation and to increase free radical formation. Altogether, these processes cause endothelial dysfunction and contribute to the impairment of organ function. A better insight into the mechanisms underlying CNI nephrotoxicity could assist in developing more targeted therapies of arterial hypertension or preventing CNI nephrotoxicity in organ transplant recipients, including heart transplantation.

## Introduction

Chronic heart failure (CHF) remains an important health issue, even in developed countries, with incidence still on the rise. CHF cannot be defined as a separate disorder as it is associated with various cardiovascular diseases that lead to impairment of diastolic or systolic left ventricular function. While untreated hypertension has been a principal contributor to CHF dating back to the 1960s, coronary artery disease has now become the major cause. Cardiomyopathy is another predominant risk factor involved in the development of CHF, especially in younger patients. CHF is considered to be a progressive medical disorder where the signs and symptoms of heart failure become more prominent with age, and is ranked among the leading causes of hospitalisation in patients over 65 years. In general population, the occurrence of CHF varies greatly and is dependent on gender, age, race and other factors, such as obesity and diabetes. Populations suffering from CHF in developed countries range from 2% to 3%. In the near future, it can be expected that this occurrence will increase, and not just in the EU (Dunlay and Roger 2014). There are reasonable explanations for this trend such as higher average life expectancy due to the improved conditions of life and therapeutic progress in medicine. Improved management of acute heart disease leads to a higher survival rate, but as evidenced by long-term outcomes the prevalence of end-stage CHF increases in these patients. Heart transplantation is considered in the event that CHF becomes so severe that it does not respond to any other therapy.

The introduction of CNIs to clinical use in the late 1970s played a major role in the advancement of transplant medicine. Cyclosporine A (CsA) has lowered rates of acute rejection and improved early graft survival. In 1984, tacrolimus (Tac), a macrolide-structure immunosuppressant was launched in Japan (Kino *et al.* 1987). Despite their positive role in development of potent immunosuppressive regimens CNIs have some limitation due their side effects. The most notable are the development of arterial hypertension and the alteration of

kidney function. The goal of this paper is to review the available literature on the pathophysiological mechanisms of CNIs that induce chronic nephrotoxicity and arterial hypertension.

## **Immunosuppressive therapy**

Immunosuppression is required in transplant recipients in order to prevent episodes of graft rejection and subsequently to reduce morbidity and mortality. Induction is effective for initial immunosuppression, which allows graft rejection to be prevented during the period when the immune response to the allograft antigen is at its most intensive. Immunosuppressive induction is used in about 50% of heart transplant centres. Interleukin-2 receptor (IL-2R) antagonists are the most frequently used induction agents in 30% of all heart transplants, whereas polyclonal anti-lymphocytic antibodies are used in 21% (Lund et al. 2015). Maintenance immunosuppression includes a combination of CNI, such as Tac or CsA, and anti-proliferative agents (most commonly mycophenolate mofetil) with or without different dosage regimens of corticosteroids.

## **Calcineurin inhibitors**

The discovery of CsA, cyclic peptide isolated from the ascomycete fungus *Tolypocladium inflatum*, represented an important step in immunosuppressive treatment. CsA was used for the first time by Calne *et al.* (1979). Thereafter, CsA became a part of the standard immunosuppressive prophylaxis, leading to a reduction in acute rejection and an improvement in one-year survival of heart transplant recipients that increased to 80% (Bolman *et al.* 1987). Tac is a macrolide immunosuppressive compound, which was isolated from the culture of *Streptomyces tsukubaensis* in Japan in 1984 (Kino *et al.* 1987) and introduced to clinical practice in the 1990s (Pirsch 1997).

The main objective of CNIs is to inhibit synthesis of IL-2 by calcineurin blockade mechanism. In T-cells, both CsA and Tac bind with high affinity to proteins known as immunophilins. CsA binds with cyclophilin and Tac binds with FK binding protein – FKBP. This immunophilin complex inhibits the phosphatase activity of calcineurin by regulating intracellular calcium transport, thereby blocking the activity of nuclear factor of activated T cells (NF-AT). Consequently, T cells do not produce IL-2 and other cytokines (IL-3, TNF  $\alpha$  and interferons). These mechanisms induced by CNIs are very similar; however, Tac seems to be 50-100 times more effective (Peters *et al.* 1993). In contrast to CsA, Tac displays further positive effect such as stimulation of the apoptotic mechanism in antigen-specific activated T-cells (Migita *et al.* 1999). Tac also diminishes mRNA expression of IL-10 and provides better protection against acute rejection (Suthanthiran 1997) and reduces anti-HLA antibody production more than CsA (Woo *et al.* 1990). In addition, it is more effective at reducing profibrotic transforming growth factor  $\beta$  (TGF  $\beta$ ) in kidney allografts (Matl *et al.* 2005). Altogether, this knowledge has led to the general practice of Tac being used as an integral part of immunosuppressive protocols in transplantation medicine.

One novel mechanism of CNIs was reported in recent studies (Faul *et al.* 2008 and Liao *et al.* 2015). This beneficial effect of CNIs on reducing proteinuria is not dependent on the inhibition of NF-AT but results from the stabilization of kidney podocytes. Therefore CsA or Tac may be helpful in treatment of nephrotic syndrome (Westhoff *et al.* 2006).

### **Side effects of calcineurin inhibitors**

Although the mechanisms underlying the effects of CsA and Tac are very similar, there are differences in their side effects. Tac treatment has been linked to the development of post-transplant diabetes (Pham *et al.* 2012) and incidence of neurological complications, such as tremors and secondary epilepsy. On the other hand, CsA induces hyperlipidaemia,

hyperuricemia, gingival hyperplasia and hirsutism more often than Tac treatment. In clinical practice, arterial hypertension and kidney dysfunction remain the most important complications induced by CNIs.

### **Pathogenesis of post-transplant arterial hypertension**

Pathophysiological mechanisms of post-transplant hypertension, which is considered a negative side effect of immunosuppressive therapy, remain unclear; CNIs apparently affect many physiological systems that play a key role in blood pressure regulation (the renin-angiotensin system, the sympathetic nervous system, the endothelin system, the nitric oxide system and the production of free radicals). After heart transplantation, the denervation causes changes in the regulation of the systemic circulation. Post-transplant hypertension can be characterized by abnormal circadian oscillation of blood pressure which increases (instead of decreasing) in the night due to increased vascular resistance (Idema *et al.* 1994). The occurrence of arterial hypertension in heart transplant patients is quite high, with about 71% in the first year after transplantation and 91% after 5 years (Lund *et al.* 2015). Hypertension can be involved in heart allograft hypertrophy (Nakata *et al.* 2000). Hypertension therapy after transplantation is complicated by major blood pressure oscillations and there is often a need to combine more anti-hypertensive drugs to control blood pressure more efficiently.

### **Nephrotoxicity**

Nephrotoxicity remains the most common and serious problem during CNI treatment. Myers *et al.* (1984) described the decline of glomerular filtration in 17 patients after heart transplantation treated with CsA over 1 year. Irreversible kidney failure was observed in two patients and biopsies confirmed focal glomerulosclerosis and tubulointerstitial injury. Renal dysfunction in patients who underwent solid organ transplantation is still frequent. Based on the International Registry of heart transplants, the prevalence of renal dysfunction is 24% at 1

year after transplantation, 49% at 5 years and 65% at 10 years after the procedure (Lund *et al.* 2015). Five to 10% of heart transplant recipients develop end-stage renal failure (Frimat *et al.* 1998). The risk of severe renal dysfunction gradually increases with increasing recipient age and recipient pre-transplant serum creatinine (Parry *et al.* 2000). Progressive renal dysfunction was also confirmed by our analysis of heart transplants. We noted that patients with serum creatinine  $\geq 150$   $\mu\text{mol/l}$  at 5 years post-transplantation had significantly worse renal function as early as 1 month and also 1 year after the procedure (Hosková *et al.* 2008). This is the result of the nephrotoxic effects of CNIs, despite the fact that there is no clear correlation between cumulative dosage of CNI and the degree of kidney dysfunction (Viklický *et al.* 1999).

Additionally, CNI treatment can induce both acute and chronic renal dysfunction. Acute effects of CNIs are presented mainly by vasoconstriction of the afferent arteriole which can occur even after the first dose is given. This leads to a decrease in glomerular filtration and an increase in serum creatinine (Andoh *et al.* 1997), followed by hypertension, hyperkalemia, tubular acidosis and enhanced sodium reabsorption with associated oliguria. These hemodynamic effects are functional changes, which are usually dose-dependent and reversible through the reduction of the CNI dose.

However, CNIs cause morphological changes in kidney tissue known as chronic nephropathy (Naesens *et al.* 2009) which can result in progressive renal insufficiency. Clinical and histological signs of nephrotoxicity are very similar during CsA or Tac administration (McCauley 1993). Typical histopathological changes in the kidney include arterial hyalinosis, tubular atrophy, interstitial fibrosis, thickening of the Bowman's capsule and focal, segmental and global glomerular sclerosis (Williams and Haragsim 2006).

## **Pathophysiological mechanisms of CNI-induced hypertension and chronic nephrotoxicity**

Findings of nephrotoxicity in early studies using CsA as an immunosuppressant launched a research into the pathophysiology of this process. Many factors are involved in CNI-induced chronic nephrotoxicity and this review summarizes each pathophysiological mechanism in the development of hypertension and renal damage (Figure 1). The major mechanisms that have been proposed are: **activation of the renin-angiotensin system** (Lee 1997, Mervaala *et al.* 1999) and the **sympathetic nervous system** (Murray *et al.* 1985), **increased production of endothelin-1** (Kohan 1997, Lanese and Conger 1993), **induction of oxidative stress** (Nishiyama *et al.* 2003) and **alteration of the NO system** (Curtis 2002, Naesens *et al.* 2009). Together these changes cause systemic and renal vasoconstriction leading to the development of endothelial dysfunction, hypertension and kidney damage (Figure 2). Besides the major mechanisms, the effect of CNIs induces other processes, such as reduced production of prostacyclin and increased synthesis of thromboxane and TGF  $\beta$  (Campistol *et al.* 2001). Some studies have indicated altered regulation of intracellular calcium (Lo Russo *et al.* 1996), magnesium deficiency (Mervaala *et al.* 1997) and decreased dopamine production in the kidney (Pestana *et al.* 1997). Finally, disruption in sodium excretion has been linked to the effect of CNI on tubular transport (Hoorn *et al.* 2011). CNI-induced oxidative stress and changes in HDL cholesterol may also affect both the cardiovascular system and kidney function (Nishiyama *et al.* 2003; Singh *et al.* 2014).

### **The renin-angiotensin system**

The renin-angiotensin system (RAS) is a signalling pathway responsible for regulating electrolytes of the body as well as water homeostasis and blood pressure. The complete scheme of the RAS cascade is demonstrated in Figure 3. The main cascade indicates that



renin is an important enzyme that cleaves plasma angiotensinogen (AGT) to angiotensin I (ANG I). Renin secretion is controlled by three following mechanisms: 1) the intrarenal baroreceptor mechanism, 2) the macula densa mechanism and 3) the activity of the sympathetic nervous system (SNS). The next step is the conversion of ANG I to ANG II by angiotensin-converting enzyme (ACE) which catalyzes this reaction (Navar *et al.* 2002).

ANG II is a potent vasoconstrictor agent, which potentiates aldosterone release and stimulates the release of catecholamine, increases the release of antidiuretic hormone in the brain and stimulates thirst. It also increases myocardial contractility, and reduces urinary losses of water. ANG II may also cause pressure-induced renal injury via its ability to induce systemic and glomerular hypertension (Siragy 2006). It also exhibits hypertrophic and proliferative effects and contributes to the progression of renal damage, thus driving the recruitment and inflammatory activation of immune cells and fibrosis (Neilson *et al.* 1993). Most of the hypertensive effects of ANG II (vasoconstriction, sodium retention) are mediated via specific receptors for angiotensin II type 1 receptor (AT<sub>1</sub>R) (Cervenka *et al.* 1999, Navar 2013, Carey 2015), which also support cell growth. Besides the kidney, AT<sub>1</sub>R are found in many other organs, including the heart (positive chronotropic and inotropic effects of ANG II on cardiomyocytes) and the brain (release of vasopressin, thirst, sympathetic activation, regulation of blood pressure). Thus, selective AT<sub>1</sub>R antagonist (ARB) drugs are used to efficiently reduce blood pressure (Navar *et al.* 2002, Carey and Siragy 2003, Carey 2015).

The vasodilatative arm partially antagonises the vasoconstrictive arm. In contrast to AT<sub>1</sub>R, ANG II type 2 receptor (AT<sub>2</sub>R) inhibits cell growth and proliferation and increases the excretion of sodium. Stimulation of AT<sub>2</sub>R causes significant vasodilation. Recent studies have investigated the role of nitric oxide (NO) in the AT<sub>2</sub>-mediated vasodilatory response to ANG II in various vessels (Dinh *et al.* 2001, Mehta and Griendling 2007). AT<sub>2</sub>R also inhibit renin synthesis (Siragy *et al.* 2005).

Angiotensin-converting enzyme 2 (ACE2) forms angiotensin 1-7 (ANG 1-7), which exerts anti-proliferative and anti-hypertensive effects. The potential imbalance between these two arms can contribute to hypertension or hypotension (Navar 2013). Stimulation of MasR by the ANG 1-7 pathway leads to activation of endothelial NO synthase. The consequent release of NO induces vasodilation (Carey 2015). The diuretic and natriuretic effects of ANG 1-7 are partially due to renal vasodilation, but may also be caused by the inhibition of sodium and water reabsorption along the nephron. An additional new RAS pathway is the alamandine/Mas-related G-protein coupled receptor (MrgD). Alamandine also induces NO-dependent vasorelaxation and its anti-fibrotic activity has been demonstrated (Gembardt *et al.* 2008).

In addition, activation of the receptor prorenin (PRR) increases plasminogen activator inhibitor 1 (PAI-1) and collagen formation, and potentially contributes to tissue fibrosis through the generation of TGF- $\beta$  (Krebs *et al.* 2007; Sihn *et al.* 2010).

### ***The role of intrarenal RAS in the regulation of renal function***

Intrarenal RAS has been described in some studies as an independent functional tissue system and characterised on the basis *in vivo* experiments. In these studies, intrarenal inhibition of RAS resulted in significant increases in renal hemodynamics and tubular excretion of sodium and water, after which all RAS components were ascertained in the kidney (Navar *et al.* 2002; Carey and Siragy 2003). Renin is not only produced by cells of the juxtaglomerular apparatus but also by proximal tubular cells. AGT is also secreted by proximal tubular cells into tubules or into the interstitial space as a source of ANG I and further converted to ANG II by ACE on the brush border of the proximal tubule (Carey and Siragy 2003). Local expression of AGT is stimulated by ANG II and this positive feedback can lead to an increase in intrarenal ANG II concentration (Navar *et al.* 2002). Furthermore,

intrarenal ANG II concentration is also enhanced by internalisation via AT1 receptor-mediated endocytosis (Navar *et al.*, 2002). Altogether, these mechanisms exceed ANG II concentration in the kidney over plasma level (Nishiyama *et al.* 2002).

### ***Activation of the renin-angiotensin system by CNIs***

Some interesting data of the interaction between CNIs and RAS have been presented over recent years (Lee 1997, Mervaala *et al.* 1999; Stilman *et al.* 1995, Hořková *et al.* 2014). The activation of RAS is initiated by direct induction of renin production and release in juxtaglomerular cells (Hoorn *et al.* 2012). A study by Prokai showed that CNIs induce renin production in the collecting duct and correspond with increased vascular endothelial growth factor (VEGF) production, which results in disproportional vessel growth (Prokai *et al.* 2016). A study by Nishiyama and colleagues demonstrated that ANG II levels are increased in both plasma and kidney tissues of CsA-induced hypertensive rats (Nishiyama *et al.* 2003). However, the mechanisms by which CNIs induce ANG II production remain to be elucidated. Increased renin secretion may also be induced secondarily by the hemodynamic effect of CNI (Hansen *et al.* 1997). CsA causes intrarenal vasoconstriction, leading to a severe reduction in blood supply to nephrons. In addition, cyclosporine stimulates platelet-derived growth factor (PDGF) (Shehata *et al.* 1995) and TGF- $\beta$  in juxtaglomerular cells (Matl *et al.* 2005). Therefore, these growth factors may also mediate the renin secretory effect of CsA (Lee 1997). Taking these findings together, CNI may cause a vicious circle in which RAS activation by CNI reduces renal hemodynamics, induces local ischaemia in the kidneys, further increases renin release and potentiates nephrotoxicity (Figure 1).

Recent findings indicate that CNIs enhance intrarenal RAS activity, which in turn may increase blood pressure and further contribute to the progression of renal damage (Nishiyama

*et al.* 2003; Hořková *et al.* 2014). These studies suggest that inappropriate intrarenal activation of RAS by CNI is a crucial pathophysiological mechanism underlying chronic CNI nephrotoxicity. It also indicated that RAS blockade efficiently attenuates Tac-induced arterial hypertension and nephrotoxicity. Therefore, inhibition of RAS seems to be essential therapy for alleviation of nephrotoxic effects of chronic CNI medication. Treatment regimen should optimally involve RAS inhibition by ACEI and ARB, and combination may potentiate renoprotective effects of these antihypertensive agents (Hořková *et al.* 2014). Besides lowering of blood pressure, it seems important to substantially reduce the intrarenal enhancement of RAS activity. We aware that the current recommendation of the dual RAS blockade in human remains questionable particularly in diabetic patients for their uncertainty concerning adverse effects and outcomes that may limit applicability to clinical practice (Mancia *et al.* 2013).

### **The sympathetic nervous system**

The SNS plays an important role in the regulation of arterial pressure. Not surprisingly, increased sympathetic nervous system activity has been implicated as a primary precursor of hypertension in both humans and animal models (Esler *et al.* 2003; Ameer *et al.* 2014). Elevated sympathetic activity accelerates the development of hypertension through sympathetically-mediated vasoconstriction and increased tubular sodium reabsorption. Cardiac sympathetic stimulation also contributes to the development of left ventricular hypertrophy. The baroreceptor system opposes increases and decreases in arterial pressure. The primary purpose of the arterial baroreflex is to keep blood pressure close to a particular set point over a relatively short period of time. Overactivity of sympathetic nerves plays an important role in the pathogenesis of hypertension, heart failure and the development of chronic kidney disease (DiBona 2004, Grisk *et al.* 2004, Kobuchi *et al.* 2014). Sympathetic

nerve fibres entering the kidneys terminate in the vascular wall of the juxtaglomerular apparatus and in the renal tubules. Activation of sympathetic nerves in the kidneys increases tubular sodium reabsorption, renin release and renal vascular resistance (DiBona 2004), leading to a subsequent reduction in renal haemodynamics associated with the impairment of renal function.

### ***Activation of the sympathetic nervous system by CNIs***

Increased sympathetic nervous activity is also involved in the pathogenesis of CNI-induced arterial hypertension and nephrotoxicity (Scherrer *et al.* 1990). CNIs modulate glutaminergic neurotransmission in rat cortical neurons through a presynaptic mechanism (Victor *et al.* 1995). Based on available evidence, CsA increases sympathetic nervous activity either by direct central postsynaptic excitation mediated by the modulation of glutaminergic neurotransmission (Grassi 2009) or by increased renal efferent signalling through cyclosporine-induced vasoconstriction of the renal vascular bed, thereby leading to increased central sympathetic outflow (Zhang *et al.* 2000). The sympathetic renal nerves also richly innervate the proximal tubule and thus, play an important role in the regulation of proximal tubule sodium reabsorption (Quan and Baum 2001). Accordingly, enhanced sodium retention and increased plasma volume contribute to development of hypertension. Therefore beta-blockers display the efficient attenuation of the enhanced sympathetic activity during CNI medication.

### **The endothelin system**

Endothelins (ETs) and their receptors are closely involved in physiological regulation of blood pressure and sodium homeostasis. ETs directly regulate cardiac output, the

peripheral and central nervous systems, renal sodium and water excretion, as well as systemic vascular resistance. Endothelin-1 (ET-1), a peptide derived from endothelial cells with high vasoconstrictor activity, was discovered in 1988 (Yanagisawa *et al.* 1988). Release of ET-1 is stimulated by a variety of endogenous substances, such as ANG II, antidiuretic hormone, thrombin and cytokines. The production of ET-1 is inhibited by the release of prostacyclin, atrial natriuretic peptide and NO. Thus, ET-1 plays a major role in the functional and structural changes observed in arterial and pulmonary hypertension, glomerulosclerosis, atherosclerosis and heart failure. ET-1 interacts with the specific endothelin receptor types A and B. In the vasculature, ET<sub>A</sub> receptors are found predominantly in smooth muscle cells, whereas ET<sub>B</sub> receptors are localised in endothelial cells and, to some extent, in smooth muscle cells, macrophages and renal tubules (Kohan *et al.* 2011).

There are opposing actions to the ET<sub>A</sub> and ET<sub>B</sub> receptors. Activation of ET<sub>A</sub> induces long-lasting vasoconstriction as well as cell proliferation in different tissues and fibrosis. In contrast, activation of endothelial ET<sub>B</sub> receptor stimulates the release of NO and prostacyclin and, at the beginning, leads to vasodilatation. ET<sub>B</sub> receptor also prevents apoptosis and mediates pulmonary clearance of circulating ET-1 and the reuptake of ET-1 by endothelial cells (Lüscher *et al.* 2000).

### ***The role of endothelin in renal physiology***

Increased expression of ET-1 has also been observed in the kidneys (Kitamura *et al.* 1989). ET-1 regulates renal vascular resistance through direct interaction with the agonists, the ET<sub>A</sub> and ET<sub>B</sub> receptors, leading to modulation of afferent and efferent arteriolar resistance. In addition, ET-1 increases intracellular Ca<sup>2+</sup> in pre-glomerular smooth muscle cells, leading to renal microvascular vasoconstriction. ET-1 is also an important regulator of renal sodium and water excretion. Finally, ET-1 plays an important role in the development of

proteinuria, fibrosis, cell proliferation and inflammation. An interesting aspect of ETs activity is that can be considered a hallmark of proteinuric renal diseases as it precedes the development of glomerulosclerosis (Barton *et al.* 2012). Podocyte cells synthesise ET-1 and podocytes express both ET<sub>A</sub> and ET<sub>B</sub> receptors. Production of renal ET-1 is increased in diabetes mellitus, obesity, autoimmune diseases, oxidative stress and NO deficiency (Barton and Yanagisawa 2008). Another study demonstrated that local ET-1 levels increase in the vascular wall during hypertension (Dhaun *et al.* 2008). The nature of this pathophysiological regulation is complex and probably depends on the degree of renal damage. It appears to be associated with increased ET-1 in the kidneys (Kohan 1997).

### ***Activation of the endothelin system by CNIs***

Enhanced ET-1 expression may be a natural consequence of heart transplantation. Furthermore, CNIs can also induce the production of endogenous ET-1. Preclinical trials have shown that CNI treatment induces ET-1 synthesis by both endothelial cells and proximal tubular cells in cultures (Bunchman *et al.* 1991). Indeed, one human study has also demonstrated that CNI induces plasma ET-1 which is associated with higher blood pressure, renal dysfunction and the progression of renal injury (Cauduro *et al.* 2005). Endothelial damage leads to activation of the coagulation cascade and stimulates platelet-derived growth factor and local production of cytokines. ET-1 induces the expression of TGF- $\beta$ , an important cytokine of fibrogenesis (Hutchinson 1999). TGF- $\beta$ 1 is a key profibrogenic cytokine associated with chronic allograft nephropathy (Viklický *et al.* 2003). However, the therapeutic use of ET receptors inhibition remains uncertain.

## **The nitric oxide system**

Nitric oxide (NO) is the most important mediator of vasodilation. It is released during the conversion of L-arginine to L-citrulline by NO synthases (NOS) (Wink and Mitchell 1998). As a radical, NO diffuses through cell membranes and activates intracellular guanylyl cyclase. In the smooth muscle cells, production of cyclic guanosine monophosphate (cGMP) lowers cellular calcium levels, leading to vasodilation (Loscalzo 1995). NO also inhibits apoptotic signals (programmed cell death) via cGMP and G kinase. In immune reactions, NO plays a part in non-specific defence mechanisms, induces interferon production and regulates phagocytosis and inflammation reactions.

In the cardiovascular system, NO is significantly involved in the regulation of vascular tonus and blood pressure, affects renal hemodynamics and tubular function (Wilcox 2005), and plays a role in the gastrointestinal tract by relaxing smooth muscle and functions of the mucosal immune system. NO-induced vasodilation in the corpus cavernosum causes erection. Furthermore, it inhibits the adhesion and aggregation of thrombocytes and leucocyte activation and proliferation. In CNS, NO modulates structural morphogenesis of the brain and synapsis and neurotransmitter release. It also plays a role as a mediator of pain.

### ***The role of NO in the regulation of blood pressure and renal function***

The important role of the NO system has been shown in the regulation of cardiovascular and renal function (Majid and Navar 2001, Modlinger *et al.* 2004). Besides its potent vasodilatory effects, NO also influences sodium tubular transport and thus also affects blood pressure. Furthermore, its properties antagonize many endogenous vasoconstrictors, particularly ANG II. In the kidneys, all NOS isoforms are present, but their role in NO



production remains unclear. Pharmacological inhibition of particular NOS has revealed some of their specific roles in the regulation of renal function. Non-selective systemic NOS inhibition leads to increases in blood pressure and vascular resistance. It also alters sodium reabsorption, most likely due to enhanced sympathetic activity (Liu and Barajas 1998). On the other hand, selective NOS inhibition in the kidneys suggests that NOS isoforms may be differently involved in many pathophysiological processes. In general, lacking NO production and reduced NO bioavailability markedly impairs renal function and increases the susceptibility to organ damage induced by enhanced activity of vasoconstrictors.

### ***Inhibition of the NO system by CNIs***

Another pathophysiological mechanism of CNI-induced nephrotoxicity appears to be direct vascular effect which leads to endothelial dysfunction. However, the direct link is still unknown. It is likely that CNIs alter the functions of all NOS isoforms by several different mechanisms (Naesens *et al.* 2009) and thus, reducing NO production. This decreased NO bioavailability could lead to an inadequate vasodilation to unopposed vasoconstriction, which is a main mechanism of CNI-induced alteration of organ hemodynamics, including the kidney. Other possibility could be a lower NO availability due to other radicals during oxidative stress and tissue damage processes, such as ischemic-reperfusion injury in renal tubules leading to local apoptosis (Hortelano *et al.* 2000). It has been shown that supplementation of NO donors displays some protective effects against CNI-induced nephrotoxicity (Lopau *et al.* 2000, Mansour *et al.* 2002). Taken together NO system is significantly involved in the renoprotection and its impairment markedly contributes to the progression of CNI-induced hypertension and nephrotoxicity. Therefore antihypertensive treatment that positively affects NO system, particularly by increases of NO bioavailability, may exhibit better protection against negative effects of CNIs.

## Oxidative stress

Living cells constantly generate free radicals throughout many metabolic pathways. Radicals can be classified as reactive oxygen species (ROS) and reactive nitrogen species (RNS). They form a part of the natural defence system and have important functions, especially in phagocytosis and apoptosis. The most important radicals are superoxide ( $O_2^-$ ) and the hydroxyl radical ( $OH^\cdot$ ). Hydrogen peroxide ( $H_2O_2$ ) is not radical but quickly oxidizes to radicals. Importantly, NO is also present in living cells as a radical and can be transformed to other RNS. Production of radicals needs to be controlled by several antioxidative systems to maintain balance and protect other cells. Oxidative stress has been implicated in the pathophysiology of many disorders and diseases, such as cardiovascular disorders, including arterial hypertension (Wilcox 2005; Pacher *et al.* 2007) and atherosclerosis. It is also involved in damaging cells and is associated with ageing, inflammatory processes, cancer growth and the development of neurodegenerative diseases. An important role of ROS has also been demonstrated in reperfusion, where mitochondria display enhanced oxygen consumption. This active metabolism can cause an accumulation of ROS and thus a risk of irreversible oxidative injury.

Overall, the role of ROS as an omnipresent product of metabolic processes has been studied extensively. There is mounting evidence that ROS can directly influence organ function. In the kidneys, ROS exhibit direct vasoconstriction and also alter sodium reabsorption in the tubules. Therefore, they may alter blood pressure regulation and enhance the accumulation of  $O_2^-$  in the kidneys. In this way, ROS appear to contribute to development of hypertension (Kopkan and Červenka 2009). These mechanisms have been described in many studies that indicate important interactions between ANG II, NO and  $O_2^-$  (Wilcox 2005). Enhanced  $O_2^-$  activity diminishes NO bioavailability in tissues, leading increased

vascular resistance, lowering of GFR and sodium retention. All these processes contribute to development of arterial hypertension.

### ***Activation of ROS production by CNIs***

Zhong *et al.* (1998) showed that CsA administration led to the enhanced activity of ROS. Another clinical study also indicates that plasma levels of H<sub>2</sub>O<sub>2</sub> in kidney transplant patients treated with CNIs significantly increase (Calo *et al.* 2002). In a subsequent study by Nishiyama *et al.* (2003), clear evidence was provided to indicate that activation of RAS and ROS is involved in CsA-induced hypertension. Both RAS inhibition by the AT<sub>1</sub> receptor blocker and administration of the antioxidant 4-hydroxy-TEMPOL reduced oxidative stress and prevented development of hypertension in this model. The positive effect of RAS inhibition on reducing ROS production in transplant patients was suggested by Kidokoro *et al.* (2012). This study also indicated that overexpression of AT<sub>1</sub>R in the kidneys may contribute to CNI-induced nephrotoxicity. Thus lowering of oxidative stress induced by CNI should be also one of the main targets of antihypertensive treatment to provide substantial organoprotection.

### **Renal sodium transport systems**

One of the major kidney functions is continuous regulation of ion and water homeostasis. Sodium transport and its excretion in all parts of the nephron are especially important for maintenance of extracellular fluid volume and thus, for blood pressure regulation (Hoorn *et al.* 2012). The main sodium transport mechanisms are as follows: 1) in the proximal tubule, a high electrochemical gradient is maintained by the sodium-potassium pump (Na<sup>+</sup>-K<sup>+</sup>-ATP ase) that transports sodium from tubular fluid to the cells; 2) another

transporter, called the  $\text{Na}^+/\text{H}^+$  exchanger type 3 (NHE3), is responsible for the electroneutral exchange of sodium and hydrogen ions; 3) in the ascending limb of the loop of Henle, sodium is transported by the  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  co-transporter (NKCC2) to the site of action for loop diuretics; 4) in the distal tubule, the thiazide-sensitive  $\text{Na}^+/\text{Cl}^-$  co-transporter (NCC), is the main mechanism of sodium handling; 5) in the collecting duct, sodium channels (ENaC) regulate sodium concentrations under the control of aldosterone and vasopressin.

### ***Alterations of sodium transport mechanisms by long-term CNI administration***

Another important mechanism that contributes to development of arterial hypertension during long-term CNI administration in heart transplant patients is retention of sodium and water (Ciresi *et al.* 1992). As the kidneys play a key role in the regulation of sodium and water balance, the effects of CNIs on renal function have been shown during the early stages of rat experiments to cause vasoconstriction, particularly of the afferent arteriole. This process results in lowering of perfusion pressure and sodium filtration, and terminates with an increase in blood pressure (Kaskel *et al.* 1987). CsA has been suggested as being responsible for reducing the reabsorption of sodium in the proximal tubule most likely via alteration of NHE3 activity (Lorenz *et al.* 1999). Esteva-Font *et al.* (2007) found that cyclosporine induces exaggerated NKCC2 expression in the proximal tubules when compared with an untreated control group. Recent studies indicate enhanced activity of the thiazide-sensitive co-transporter NCC as another possible explanation for sodium retention during CNI administration (Hoorn *et al.* 2011). Therefore, thiazide diuretics could be another option in hypertensive patients treated with CNI (Colussi *et al.* 2007). Other studies have demonstrated that CNIs may also influence  $\text{Na}^+/\text{K}^+$ -ATPase; however, the results remain inconclusive (Hoorn *et al.* 2012). Therefore, all the above mechanisms can significantly alter sodium excretion and thus, contribute to the pathogenesis of hypertension induced by CNIs. Base on

that, diuretics are usually combined with other antihypertensives in treatment of chronic CNI-induced hypertension.

## **Conclusions**

Heart transplantation is now an established treatment modality in patients with end-stage heart failure when all other therapeutic options fail. The long-term outcomes of heart transplant recipients have improved considerably since the introduction of CNIs. In this review, we have outlined the pathophysiology of CNI-induced nephrotoxicity and hypertension. We have shown that CNIs lead to activation of the renin-angiotensin and endothelin systems and to increase of sympathetic nerve activity. In addition, CNIs are known to inhibit NO synthesis and NO-mediated vasodilation, and also increase free radicals and superoxide production through vasoconstriction-associated hypoxia. Increased levels of intrarenal renin and angiotensin II induced by CNI are recognized as an important mechanism that contributes to nephrotoxicity. The direct effect of CNI on the tubular epithelial cells plays a major role in the development of interstitial fibrosis. Activation of RAS is not only important in terms of its hemodynamic contribution, but it also directly promotes renal interstitial fibrosis through profibrotic effect of TGF  $\beta$ . Finally, the effect of CNI on tubular function may explain sodium retention. Continuing research of side effects of CNI and their mechanisms remains an important prerequisite for development of novel therapeutic strategies to reduce nephrotoxicity and to manage arterial hypertension.

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## **Legends of figures**

### **Figure 1**

#### **Pathophysiological mechanisms of CNI-induced hypertension and chronic nephrotoxicity**

CNI, calcineurin inhibitor; NO, nitric oxide; ROS, reactive oxygen species; SNS, sympathetic nerve activity; NCC, renal sodium chloride co-transporter; NHE3, proximal sodium hydrogen exchanger type 3; NKCC2, sodium potassium chloride co-transporter type 2; Na<sup>+</sup>-K<sup>+</sup>-ATPase, sodium-potassium pump.

### **Figure 2**

#### **The major vasoactive systems and their interactions in the development of CNI-induced endothelial dysfunction, hypertension and kidney damage**

### **Figure 3**

#### **Schematic illustration of the renin-angiotensin system cascade**

ANG I, angiotensin I; ACE, angiotensin-converting enzyme; ANG II, angiotensin II; ANG III, angiotensin III; ANG IV, angiotensin IV; ACE 2, angiotensin-converting enzyme 2; ANG-(1-7), angiotensin 1-7; AT<sub>1</sub>R, angiotensin type 1 receptor; AT<sub>2</sub>R, angiotensin type 2 receptor; AT<sub>4</sub>R, angiotensin type 4 receptor; MasR, Mas receptor (receptor for angiotensin 1-7); PRR, (pro)renin receptor; MAP kinases, mitogen-activated protein kinases; ERK,

extracellular signal-related kinase; TGF  $\beta$ , transforming growth factor  $\beta$ ; PAI-1, plasminogen activator inhibitor-1; MrgD, Mas-related G-protein coupled receptor

Figure 1

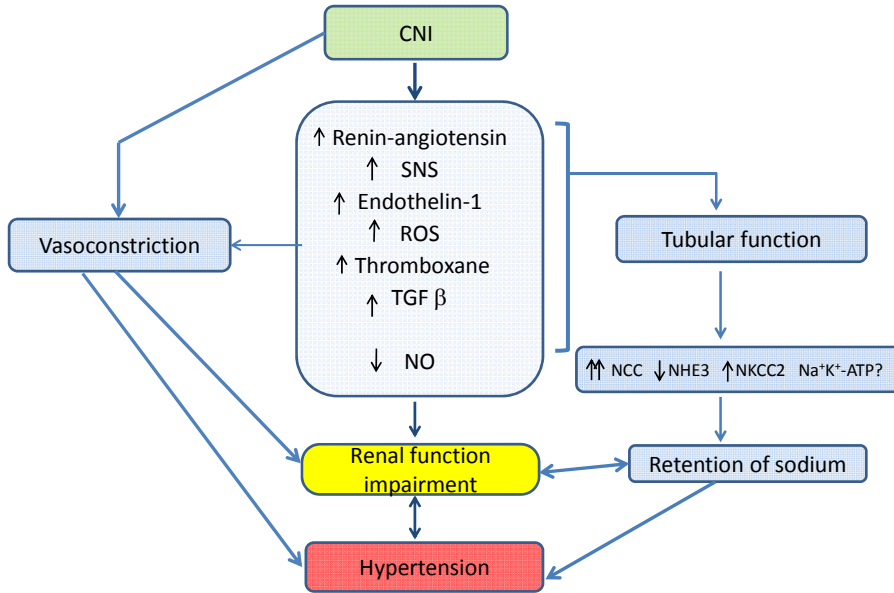


Figure 2

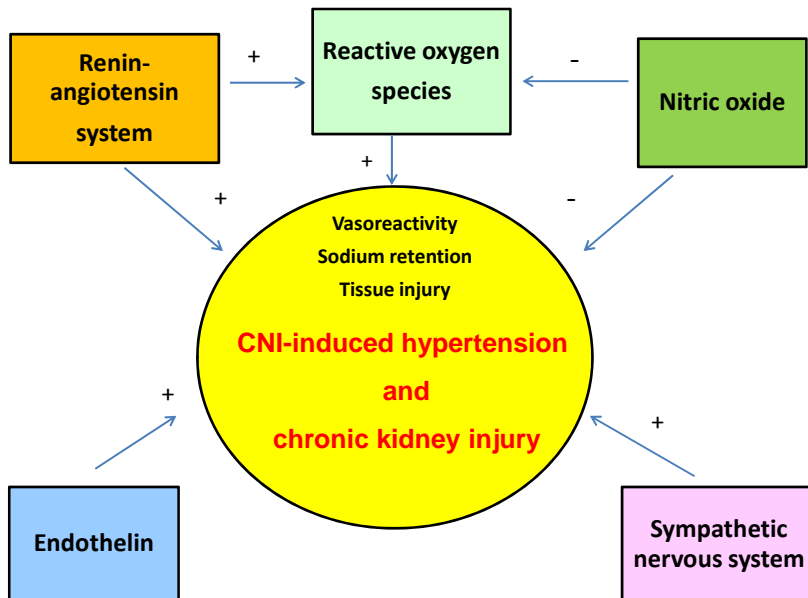


Figure 3

