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Prevention of Nephrotoxicity Induced by Cyclosporine-A: Role of Antioxidants

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ABSTRACT

Cyclosporine A (CsA) is a powerful immunosuppressive drug used to prevent allograft rejection after organ transplantation as well as in human and veterinary medicine. Unfortunately, its use is hampered by its nephrotoxic effects. The mechanisms of CsA-induced hypertension and nephrotoxicity are not clear, but several studies suggest the possible involvement of free radicals. In this review we have summarized the effect of some antioxidants that we have used in the recent years, in combination with CsA, to better understand the exact mechanism of action of CsA and to try to open new perspectives in the treatment of CsA nephrotoxicity. J. Cell. Biochem. 116: 364–369, 2015.

KEY WORDS: CYCLOSPORINE-A (CsA); REACTING OXYGEN SPECIES (ROS); ANTIOXIDANT; NEPHROTOXICITY

Cyclosporine A (CsA), a lipophilic cyclic polypeptide isolated from the fungus Tolypocladium inflatum, is a powerful immunosuppressant drug that has improved the management of transplantation and autoimmune diseases. The suppression of the activation and proliferation of T cells by CsA is due to the inhibition of the synthesis of interleukin IL-2, that leads to the suppression of secondary synthesis of various cytokines, such as IL-4, interferon-γ and granulocyte-macrophage colony stimulating factor [Wood and Lemaire, 1985]. In human, CsA administration significantly improves long term survival in case of solid organ transplantation [Ciresi et al., 1992]. In veterinary medicine, CsA is used in cats to prevent allograft rejection [Mishina et al., 1996].

In dogs to treat canine atopic dermatitis, keratoconjunctivitis sicca, perianal fistula (Morgan and Abrams, 1991; Mathews et al., 1997; Guaguère et al., 2004), and in subjects with end-stage chronic renal failure [Mathews et al., 2000]. Unfortunately, the CsA treatment shows several limitations related to its nephrotoxic effects, like the decrease of glomerular filtration rate (GFR) and hypertension as previously demonstrated in rat models [Damiano et al., 2013] as well as in clinical practice [Lee et al., 2011]. In fact, when the concentrations of CsA are higher than the therapeutic range (400–600 ng/dl), CsA toxicity may emerge. It has also been reported, in human allograft, that CsA induces necrosis and hyalinosis of smooth muscle cells in the afferent renal arterioles, isometric vacuolation of the proximal tubules (PT) [Kahan, 1987] and that such effects are reversed by lowering CsA dose. Long term CsA treatment in organ transplant recipients [Bach, 1994] and in autoimmune patients [Taler et al., 1999] increases the risk of

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hypertension. Hypertension is usually reversible after discontinuation of short-term CsA therapy [Taler et al., 1999], whereas continued treatment even at reduced doses frequently results in sustained hypertension [Schwartz et al., 1996; Sheikh-Hamad et al., 2001]. Physiological alterations have been described in several studies during CsA treatment. Among functional abnormalities, the reduction of GFR and hypertension has mainly been described. The renal effects are related to the vasoconstriction of glomerular afferent arterioles, which causes a decrease of glomerular pressure [Murray et al., 1985] and an increase in serum creatinine concentration and the decrease in creatinine clearance [Lassila et al., 2000]. Such effects are dose-dependent and reverse after short term with Csa treatment [Henny et al., 1985]. Among the histological renal damage, tubulointerstitial fibrosis and arteriopathy of afferent arterioles have been documented, such effect are dose dependent but irreversible [Andoh and Bennett, 1998]. In this review, we summarized the effect of some antioxidants used in the recent years, in combination with CsA, to better understand the exact mechanism of action of CsA and to provide new perspectives in the treatment of CsA nephrotoxicity.

MECHANISMS OF CsA-INDUCED NEPHROTOXICITY

Possible mechanisms involved in CsA-induced nephrotoxicity and hypertension, include vascular endothelial dysfunction [De Nicola et al., 1993], activation of renin-angiotensin system (RAS) [Tufro-McReddie et al., 1993], increased vasoconstriction [Murray et al., 1985] and enhanced sympathetic tone and increased synthesis of endothelins [Fogo et al., 1992]. Data suggests that sodium and water retention is associated with the development of cyclosporine-induced hypertension [Ciresi et al., 1992] and possible involvement of free radicals (Parra Cid et al., 2003) (Fig. 1).

To study the nephrotoxicity of CsA, a Sprague Dawley normotensive rat model was developed [Young et al., 1995] using a high dose of CsA (15 mg/kg/day) for 4 weeks [Capasso et al., 2008]. The morphological and functional renal abnormalities described in rat model are similar to the nephrotoxic damages observed in CsA-treated patients. We found in Sprague Dawley rats treated for 3 weeks with CsA 15 mg/kg/day as well as in rats treated for 1 week with CsA 25 mg/kg/day [Damiano et al., 2013] an increase of blood pressure, a severe decrease in GFR, an increase in Reactive Oxygen Species (ROS) production and morphological damage. We also found a decrease of absolute fluid reabsorption (Jv) in the PT, in agreement with another investigator who suggested an alteration of ion reabsorption along the tubules during the development of CsA-induced hypertension [Ciresi et al., 1992].

RENIN-ANGIOTENSIN SYSTEM AND CsA

The renin-angiotensin system (RAS) is an important regulator of blood pressure and renal function, but its role in hypertension is not clear. The most important effector of RAS is angiotensin II (Ang II) that is formed by angiotensin I (Ang I). Ang I is activated by angiotensin converting enzyme (ACE), which is mainly located on the surface of the vascular endothelium and the lung epithelium. ACE seems to be the most important enzyme for Ang II formation

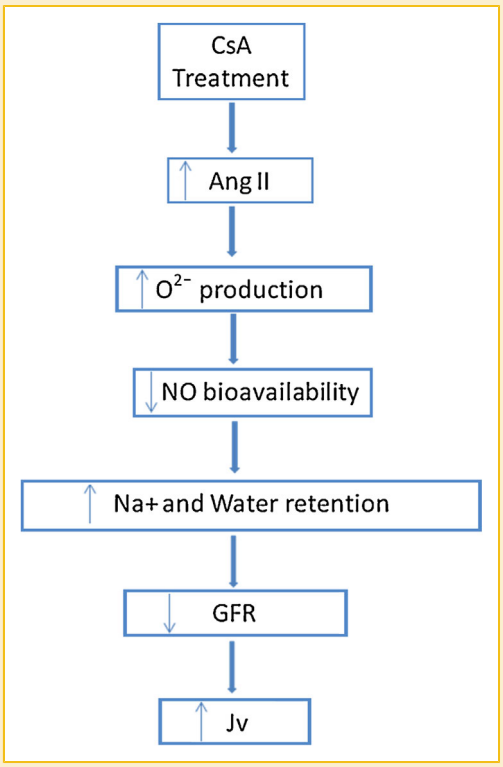

Fig. 1. Hypothesis of CsA-induced neprotoxicity. CsA could induce an increase in Ang II and O^{2-} production. This is probably the cause of reduction of NO bioavailability that induce Na+ and water retention that could induce the alteration of tubular and glomerular reabsorption.

[Okunishi et al., 1993]. In CsA-treated rats on sodium-depletion, normal-sodium [Ciresi et al., 1992] or high-sodium diets [Abassi et al., 1996] an increase in plasma renin activity (PRA) has been demonstrated [Lassila et al., 2000]. Since the CsA increases the PRA, it is possible to hypothesize that drugs suppressing RAS could reduce the CSA induced renal dysfunction, but this has not yet been fully demonstrated. It has been shown that the CsA reduced the GFR renal flow. Such reduction stimulates Ang II through vasoconstriction of the efferent glomerular arterioles and contributes to the maintenance of GFR [Murray et al., 1985; Myers et al., 1988; Mervaala et al., 1997; Lassila et al., 2000]. Thus, it is possible that the dilation of the efferent arterioles by drug suppression of RAS could restore the GFR.

OXIDATIVE STRESS AND CsA

Studies by Hall et al. (1999) reveal that free radicals are dramatically increased in rat kidney after CsA treatment. Furthermore, it has been reported that CsA induces membrane lipid peroxidation in transplant patients [Wong et al., 2002]. Several ROS are involved in CSA-induced nephrotoxicity, but the most important is superoxide (O^{2-}) which is synthesized in mitochondria by xanthine. In the kidney it is mainly produced by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) [Wilcox, 2005; Nouri et al., 2007].

The NADPH oxidase subunits in the kidney are found in the blood vessels, interstitial cells, glomeruli and tubules [Wilcox, 2005]. In

fact, an early study identified NADPH oxidase components in mesangial cells [Nava et al., 2003] and it was been demonstrated that human glomerular mesangial cells produce ROS and express p22phox, p67phox, and p47phox components of NADPH oxidase [Radeke et al., 1991] and Nox-4 [Jones et al., 1995]. Moreover, it has been shown that the outer medullary thick ascending limb (TAL) of the loop of Henle of rats expresses p40phox, p47phox, p22phox and Nox-2 [Li et al., 2002; Gorin et al., 2005; Li et al., 2002; Joshi et al., 2013]. Finally, the renal cortex of Wistar rats and spontaneously hypertensive rats present mRNA and protein expression for Nox-2, p22phox, p67phox and p47phox [Patterson et al., 1999; Chabrashvili et al., 2002; Kitiyakara et al., 2003; Huang et al., 2011]. There are several scientific studies showing that CsA treatment stimulates ROS production. In fact, an increase in renal cortical of lipid peroxidation and an increase of urinary excretion of ROS during administration of CsA has been observed [Calò et al., 2002]. In addition, the same authors reveal in hypertensive patients treated with CsA, an increase in the plasma hydroperoxide levels and an increase of mRNA expression of p22-phox, an essential NADPH oxidase component. Moreover, studies by Galle et al. (2000) reveal that incubating rat aortic rings with CsA leads to a significant increase in superoxide release. In addition, Diederich et al. (1994) demonstrate that pre-treatment with a superoxide dismutase (SOD) normalizes the impaired acetylcholine induced relaxation in arteries isolated from rats treated with CsA. These data suggest that the endothelial dysfunction induced by CsA is related to an increase of ROS production and the development of hypertension [Nishiyama et al., 2003; Damiano et al., 2013].

ANTIOXIDANTS AND CsA TREATMENT

Since it has been hypothesized that CsA toxicity could be mediated by ROS [Parra et al., 1998], in the last several years antioxidants have been tested to find a new drug that could prevent the damages induced by CsA. In the following paragraphs, we summarize recent reports on antioxidant effects on CsA treatment (Table I).

THE INFLUENCE OF THE ANTIOXIDANT HYDROCORTISONE

To improve the therapeutic effects of CsA, in several protocols, CsA is administered in association with corticosteroids. In fact, several lines of evidence suggest that the hydrocortisone (HY), a steroidal anti-inflammatory drug, is able to reduce lipid peroxidation induced in rat by ligated loop of the distal ileum or in the rat hyppocampus under stress condition [Tolstuckima et al., 1999]. The immunosuppressive activity of HY is due to its ability to induce apoptosis of T lymphocytes by activation of lysis genes or by the repression of the expression of genes involved in proliferation and cell growth [Wyllie, 1980; Cohen, 1991; Jeon et al., 2005]. Recently we have analyzed [Ciarcia et al., 2012] in the kidney tissue, the effects of CsA used alone or in association with HY by in vivo experiments. We have evaluated the lipid peroxidation by assaying the Malondialdheyde (MDA) production by means of the thiobarbituric acid test and we have found that CsA treatment increases MDA levels while HY is able to reduce the CsA activity. We have also analyzed the catalase, the superoxide dismutase and the glutathion peroxidase levels and have found that HY reduced the nephrotoxic effects induced by CsA [Ciarcia et al., 2012]. These data, together, demonstrate that, in rat kidney, CsA toxicity is due to an oxidative stress overload and that the HY reduces lipid peroxidation and consequently inhibits the toxicity induced by CsA. Unfortunately, HY use is limited by its chronic toxicity such as the suppression of body weight gain and food intake. [Ciarcia et al., 2012].

THE INFLUENCE OF THE ANTIOXIDANT VITAMIN E (Vit E)

Studies by De Arriba et al. (2013) reveal that glomeruli of rats treated with CsA have an increase of ROS synthesis. This increase is also observed in cultures rat mesangial cells incubated with CsA. They have shown that pre-treatment with the antioxidant vitamin E inhibits cellular damage. One of the main sources of intracellular ROS is mitochondria. Studies by De Arriba et al. (2013) reveal the specific production of O^{2-} by mitochondria in LLC-PK1 cells using Mitochondrial Superoxide Indicator (MitoSOX Red). They have found that pre-treatment with vitamin E inhibit the mitochondrial synthesis of O^{2-} suggesting that the antioxidant can avoid nephrotoxic effects of CsA by scavenging O^{2-}. These data are supported by other authors that have proved that selective inhibitors of mitochondrial electron transport decrease the generation of ROS induced by CsA in MDCK cells [Jeon et al., 2005]. These findings are in agreement with the results obtained in an our previous study in which we measured ROS production by the dichlorofluorescein (DHE) and Thiobarbituric Acid Reactive Substances (TBARS) assays finding a decrease in ROS and TBARS by vitamin E treatment [Ciarcia et al., 2012]. Unfortunately, there are not enough data in the

TABLE I. Summary of the Effects of Each Antioxidant on CsA Nephrotoxicity.

Antioxidant	ROS levels	Histological damage	GFR	Jv	BP
Hydrocortisone[a]	Restored	Partially restored	No data available	No data available	No data available
Vitamin E[b,c,d]	Restored	Partially restored	No effect	No data available	No data available
DOPET[e]	Restored	Partially restored	No data available	No data available	No effect
rMnSOD[f]	Restored	Partially restored	Partially restored	No data available	No effect

[a]Ciarcia et al., 2012.
[b]Andres and Cascales, 2002.
[c]Parra et al., 1998.
[d]Bach, 1994.
[e]Capasso et al., 2008.
[f]Mancini et al., 2006.

literature about the protective effect of vitamin E on the kidney functions. There are evidences suggesting that vitamin E has not significant effects against the CsA-induced reduction in GFR [Bárány et al., 2001].

THE INFLUENCE OF THE ANTIOXIDANT HYDROXYTYROSOL (DOPET)

The natural antioxidant phenol hydroxytyrosol (DOPET), present in high concentrations in extra virgin olive oil, was tested in order to verify its ability to reduce CsA-induced nephrotoxicity, based on the high bioavailability, the high scavenging power and the in vivo low toxicity [Bárány et al., 2001; D′Angelo et al., 2001; Capasso et al., 2008]. We have shown that DOPET reduced the CsA-induced oxidative stress in cells of aorta and in renal artery during DHE experiments [Galletti et al., 2005], but it was unable to prevent CsA induced hemodynamic effects. We have observed hypertension as well as a 50% decrease in GFR in rats treated for 21 days with CsA [Capasso et al., 2008], but we have not observed any protective effect on GFR and blood pressure when the rats were treated with CsA plus DOPET. These data suggest that the hemodynamic alteration and the hypertension are not necessarily related to the increase of free radical. It is possible that other underlying mechanisms, such as artheriolopathy, could act on renal failure and hypertension. This interpretation is in contrast with the data reported in the literature demonstrating that some antioxidants, like vitamin E [Parra et al., 1998] and licopene [Atessahin et al., 2007] are able to reduce oxidative stress and renal function at the same time. However, it must be considered that such compounds, besides their antioxidant activity, have a key role in the modulation of some enzymatic activities and in alteration of gene expression (Andrès and Cascales, 2002; Siler et al., 2004). Therefore, further investigations are required in order to clarify their activity in renal hemodynamic. For example, it would be interesting to assess the effects on the kidney of a higher dose of DOPET on renal function during treatment with CsA.

THE INFLUENCE OF THE ANTIOXIDANT MITOCHONDRIAL RECOMBINANT MANGANESE CONTAINING SUPEROXIDE DISMUTASE (rMnSOD)

MnSOD is superoxide dismutase (SOD) family member, mainly located in the mitochondrial matrix [Weisiger and Fridovich, 1973; Okado-Matsumoto and Fridovich, 2001; Zelko et al., 2002; Holley et al., 2012] encoded by different genes. It has anticancer properties both in vivo and in vitro [Ridnour et al., 2004; Damiano et al., 2013] directly acting on the growth rate, invasiveness, anchorage-independent growth, etc. of cancer cells. A new recombinant MnSOD (rMnSOD) has been isolated by our group from a human pleiomorphic liposarcoma cell line [Mancini et al., 2006]. While MnSOD is generally localized in the mitochondrial matrix, the rMnSOD is mainly secreted into the media. Since it has strong antioxidant activity, we evaluated the effects of rMnSOD on CsA nephrotoxicity and we have found that, with respect to DOPET, rMnSOD is more effective on renal hemodynamic damage induced by CsA. We have shown, in rats treated with rMnSOD plus CsA, a good restoration of ROS production and in the GFR but we have not found a restoration of blood pressure [Damiano et al., 2013].

In conclusion, our data indicate that rMnSOD is able to prevent arterial and renal oxidative stress and the reduction in the GFR consequent to CsA administration. In addition, renal morphology was partially improved, in fact, the lesions were mainly tubular, interstitial and arterioral. It would be interesting to perform a longer treatment (3 weeks rather than 1 week) to see if there is an effect on the blood pressure.

CONCLUSION AND FUTURE PERSPECTIVES

The data presented herein show that the mechanism of nephrotoxicity induced by CsA is strongly influenced by oxidative stress, but the different antioxidant compounds used, while being able to restore the normal ROS levels, do not produce therapeutic effect on renal hemodynamic. We have demonstrated that only the rMnSOD is able to restore the GFR, but we have not found any effect on blood pressure [Damiano et al., 2013]. This lack of efficacy is probably related to the mechanism of action of the antioxidants used that act on ROS production in general. During CsA treatment, we have demonstrated a GFR decrease, by clearance of inulin, and we have shown that the decrease of absolute fluid reabsorption in proximal tubule (PT), measured by micropuncture experiments, is related to an increase in O^{2-} measured by DHE assay [Damiano et al., 2013]. We hypothesize that the decrease of GFR is linked with the increase of O^{2-} that reduce the availability of Nitric oxide (NO), which could be the cause of glomerular vasoconstriction. It is possible that blocking the activity of NADPH oxidase, a simultaneous recovery of GFR and hypertension could be observed as a result of the increased level of available NO. To prevent the reduction of NO, a good drug candidate might be 4′-Hydroxy-3′-methoxyacetophenone (Apocynin), a more specific inhibitor of O^{2-} production [Panico et al., 2009]. Apocynin prevents the assembly of the NADPH oxidase to the cell membrane thereby blocking the production of superoxide (Stolk et al., 1994) and limits the amount of superoxide available for the binding with NO. Therefore, according to its characteristics, Apocynin might be useful to reduce the toxic effect of the CSA and studies in our laboratories are in progress to test this hypothesis.

In conclusion, the exact mechanism of nephrotoxicity induced by CsA remains unclear and more experiments are necessary to investigate these effects. Thus, the use of specific antioxidants of new generation, like rMnSOD and Apocynin, could reduce the nephrotoxic effect induced by CsA and open new perspectives in the treatment of CsA nephrotoxicity.

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