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Cyclosporine A nephrotoxicity: Role of oxidative stress

Nefrotoksyczność cyklosporyny A: Rola stresu oksydacyjnego

INTRODUCTION

Cyclosporine A (CsA) is a calcineurin inhibitor which has remained for many years a crucial immunosuppressant with a major therapeutic role in a solid organ transplantation and in various immunological diseases. The main adverse effect of CsA is nephrotoxicity. CsA causes acute renal damage as well as a chronic tubulointerstitial nephropathy characterized by tubular atrophy, vascular injury, glomerulosclerosis and interstitial fibrosis. Although the mechanisms of CsA dependent nephrotoxicity are still not fully understood, several vasoactive mediators have been proposed as being responsible for the derangement of renal function induced by the drug. The renin-angiotensin system, endothelin and eicosanoids have been considered as potential mediators of CsA-related kidney dysfunction.

Recently reactive oxygen species (ROS) release and consequently oxidative stress have been proposed as an alternate source of CsA-dependent kidney damage [9–11, 15]

CsA has been shown to cause imbalance of the cellular oxidative status as a result of excessive ROS formation. The production of ROS by CsA is postulated to be due to action of CsA as an uncoupler and inhibitor of the mitochondrial electron transport system, drug's action on NADPH oxidase or xanthine oxidase, the result of decreased cellular antioxidant system or CsA metabolism by cytochrome P450 [15]. It is suggested that these ROS can attack unsaturated bonds of membrane lipids in an autocatalytic process and result in lipid peroxidation. The oxidative breakdown of membrane polyunsaturated fatty acids causes increased cellular membrane permeability, with subsequent alterations of ionic gradients and disruption of several membrane functions and metabolic

processes [22]. The increased levels of ROS and lipid peroxidation products following CsA have been reported in many experimental studies *in vitro* and *in vivo* [3,4,13,26,30]. Nishida et al. [21] demonstrated an increase in superoxide anion radical (O2- *) and hydrogen peroxide (H2O2) level following CsA administration.

Mitochondria are one of the main physiologic sources of ROS, especially O_2-*. In chronic nephrotoxicity, CsA-induced increase in O_2^-* was demonstrated in renal tubular and endothelial cells [9,23,29]. Increase in mitochondrial Ca^{2+} ions that follows CsA administration is the main mechanism that triggers the reaction [25,26,28]. Increased production of ROS may take place on different levels of respiratory chain [22,25,29]. Moreover, Vetter et al. [29] demonstrated that CsA-induced O_2^-* release in tubular proximal cells depends on the presence of NADPH nucleotide and is produced by NADPH oxidase. Mitochondria also have a complex antioxidant system that can detoxify these ROS, among which there are enzymes (dismutase superoxide, gluthatione peroxidase, catalase, gluthatione reductase, NADP trans-hydrogenase) and other molecules (gluthatione, NAD(P)H, and vitamins E and C) that combined constitute and effective antioxidant system. The action of the components of this system is integrated so that the O_2^-* released in the respiratory chain is metabolized by the mitochondrial dismutase superoxide (SOD-Mn) to hydrogen peroxide, and this is detoxified by intra- and/or extramitochondrial gluthatione peroxidase. CsA produces an increase in mitochondrial O_2^-* and this increase parallels the oxidation of NADPH, an essential molecule in the maintenance of the reduced state of complex I of the mitochondrial respiratory chain, which decrease is related to different models of cellular death [17,18,23,29].

ROS are highly reactive and can react with many intracellular molecules, mainly unsaturated fatty acids and transmembrane proteins triggering lipid peroxidation and protein damage. The oxidation of these molecules causes increased cellular membrane permeability, with consequent alteration of ionic gradients and disruption of several membrane functions and metabolic processes. As a consequence of CsA-dependend lipid peroxidation an increase malondialdehyde (MDA) concentration in endothelial, mesangial or tubular epithelial cells is observed [3,22,28]. It has been demonstrated that lipid peroxidation induced by CsA was dose-dependent and paralleled the renal functional alterations [22] as well as structural damage [28]. Among the consequences of O_2^-* release increased oxidation of cardiolipin is noted. Cardiolipin (CL) is the main lipid constituent of inner mitochondrial membrane and contributes to its stabilization by interacting with the complexes and proteins of the respiratory chain. Its oxidation can have important consequences on mitochondrial physiology and structure. It affects several complexes of the mitochondrial respiratory chain, altering the electronic flow through them. The electrochemical gradient important for ATP synthesis is not generated and the cellular energy metabolism will be compromised [9].

Another important effect is derived from the fact that oxidized CL binds with less affinity to cytochrome *c*, thus favoring its release to the intermembranous space and to the cytosol. This suggests that CsA promotes so-called mitochondrial permeability transition pores (MPTP) opening [9]. These pores are created at sites of contact of the inner and outer mitochondrial membranes. Their opening may condition the release of mitochondrial pro-apoptotic factors such as cytochrome c and promote cell death [9,17]. It also will promote further interruption of the electron transfer within the respiratory chain and its decoupling.

An efficient antioxidant defense system operates to combat the production of free radicals. Among most important antioxidants *in vivo* are catalase, superoxide dismutase reduced gluthatione

peroxidase (GSH-Px) and GSH, all found in relatively high concentrations in the kidney [5]. It plays a pivotal role in the protection of cells against oxidative stress and detoxification of xenobiotics, including CsA [26]. Together with gluthatione peroxidase it converts H_2O_2 to non-toxic products, thus maintaining the integrity of the mitochondria and cell membranes [10, 26]. A significant decrease in level of renal reduced glutathione (GSH) after CsA administration was observed in many studies [3,4,10,28]. Inhibition of enzymes kipping GSH in reduced stage: gluthatione reductase (GR), glucose-6-phosphate dehydrogenase (G6PD) and glutathione S-transferase (GST) was observed after CsA administration [28]. Oxidized form of glutathione (GSSG), formed from the reaction of GPx, is subsequently reduced back to GSH at the expense of NADPH by GR, which is an important enzyme for maintaining the intracellular level of GSH. The decreased activity of GR may be due to depletion of GSH and NADPH levels. G6PD is not directly involved in GSH synthesis, but is the most important cellular source of NADPH which is utilized by GSH reductase to reduce GSSG to GSH. Amudha et al. [3] and Capasso et al. [7] show decreased renal GSH, increased GSSG and decreased GSH/GSSG ratio in rats treated chronically with CsA.

Oxidative stress can promote the formation of variety of vasoactive mediators that effect renal function directly by causing renal vasoconstriction or decreasing the glomerular capillary ultrafiltration co-efficient, thus reducing the glomerular filtration rate [3,10,24]. CsA-induced ROS release influences the eicosanoids metabolism in the kidney and increases tromboxane A (TXA) and its metabolite tromboxan B_2 (TXB_2) synthesis, known important vasoconstrictors [19,22]. Ischemic damages due to hypoperfusion: tubular atrophy and interstitial fibrosis or progressive glomerular sclerosis, are, at least partially triggered by oxidative stress in the course of Cs-A induced nephrotoxicity.

Another important consequence of ROS relief in the kidney is associated with interstitial fibrosis. Iglesias-deLaCruz et al. [12] demonstrated increased production of collagen I, collagen IV and fibronectin by mesangial cells treated with H_2O_2. Akool et al. [1] documented that Cs-A induced ROS activate TGF beta gene in mesangial cells. TGF beta is an important fibrogenic growth factor that promotes interstitial fibrosis and glomerular sclerosis in the kidney.

Another radical of biological importance is the nitric oxide (NO). When NO and superoxide are produced simultaneously, they react with each other to form the highly reactive peroxynitrite ($ONOO^-$), which leads to the onset of various damages [16]. CsA-induced increase in $ONOO^-$ in the kidney was documented in many experimental studies [3,8,14,27]. Navarro-Antolin et al. [20] demonstrated that CsA – induced nephrotoxicity is associated with induction of iNOS (inducible nitric oxide synthase) and nitrosative stress leading to the formation of $ONOO^-$ which mediates many of the cytotoxic effects to cellular thiols, lipids, proteins and DNA. One of the effects of these processes is cell apoptosis. Increased expression of proapoptotic protein p53 triggered by reactive nitrogen species was noted by Amore et al. [2] and Jennings et al. [14] in renal endothelial cells.

The protective role of antioxidants against Cs-A induced nephrotoxicity has been demonstrated in many studies. The use of different antioxidants or free radical scavengers such as lazariod, vitamin E, vitamin C, melatonin, trimetazidine, carvedilol and N-acetylcysteine as well as the minerals selenium and zinc, xanthine oxidase inhibition by allopurinol and viral delivery of superoxide dismutase genes consistently resulted in the improvement of renal CsA adverse effects [6,7,22,24]. Two principle mechanisms of action have been proposed. The first is chain - breaking mechanism, by which the

antioxidant donates an electron to the Peroxy radical of the fatty acid, thus stopping the propagation steps in lipid peroxidation. The second mechanism involves removal of radical species initiators by quenching chain-initiating catalyst [24]. In recent years, many plant-derived substances that are classified as polyphenols are becoming increasingly known for their various biological effects, particularly antioxidant and free radical scavenging activities [22,24]. The polyphenols have been reported to act as chain-breaking antioxidant by their ability to donate hydrogen atoms, to inhibit free radical formation by chelating transition metal ions, to act as co-antioxidant by facilitating antioxidant activity of other compounds, and to modulate signal transduction pathways and gene expression through their reducing properties [24]. There was also evidence demonstrating nephroprotective effect of these phytochemicals in several experimental models associated with oxidative stress, including nephrotoxicity induced by CsA. Polyphenolic compounds such as curcumin, quercetin, catechin, tea polyphenol, resveratol and provinol have been shown to attenuate the renal dysfunction, improve renal morphology, increase the antioxidant enzyme activity and content, decrease lipid peroxidation and reactive oxygen species in CsA nephrotoxicity [4,24,28,30].

In summary, Cs-A induced kidney dysfunction and morphological damage has multifactorial pathogenesis. CsA nephrotoxicity is strongly associated with imbalanced red-ox state and oxidative stress in renal tubular, endothelial and glomerular cells. CsA-induced ROS release in the kidney is accompanied be development of morphologic changes including vascular injury with ischemic damage, interstitial fibrosis, glomerular sclerosis, tubular atrophy and cell apoptosis.

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SUMMARY

Cyclosporine A (CsA) is a calcineurin inhibitor which has remained for many years a crucial immunosuppressant with a major therapeutic role in a solid organ transplantation and in various immunological diseases. The main adverse effect of CsA is nephrotoxicity. Cs-A induced kidney dysfunction and morphological damage has multifactorial pathogenesis. The renin-angiotensin system, endothelin and eicosanoids have been considered as potential mediators of CsA-related kidney dysfunction. Recently oxidative stress and reactive oxygen species (ROS) release have been proposed as an alternate source of CsA-dependent kidney damage. CsA nephrotoxicity is associated with imbalanced red-ox state and oxidative stress in renal tubular, endothelial and glomerular cells. CsA-induced ROS release in the kidney is accompanied be development of morphologic changes including vascular injury with ischemic damage, interstitial fibrosis, progressive glomerular sclerosis, tubular atrophy and cell apoptosis.

Keywords: Cyclosporine A, nephrotoxicity, oxidative stress, mitochondria

STRESZCZENIE

Cyklosporyna A jest inhibitorem kalcyneuryny, jednym z podstawowych leków stosowanych w terapii immunosupresyjnej po przeszczepie narządów czy szeregu chorób immunologicznych. Głównym efektem ubocznym jej stosowania jest nefrotoksyczność. W patogenezie uszkodzenia nerki przez CsA bierze udział wiele mechanizmów. Do mediatorów zaburzeń funkcji nerek i uszkodzeń morfologicznych należą min. układ renina-angiotensyna-aldosteron, produkcja wewnątrznerkowych eikozanoidów, czy endotelina. Istotne znaczenie w uszkodzeniu narządu przez cyklosporynę przypisuje się mechanizmom stresu oksydacyjnego i uwalnianym w nadmiarze wolnym rodnikom tlenowym. Procesy te zachodzą w komórkach nabłonkowych kanalików nerkowych, w komórkach śródbłonka naczyń czy w komórkach kłębuszków nerkowych. Biorą udział w zaburzeniach funkcji nerek oraz w powstawaniu uszkodzeń morfologicznych takich jak zmiany naczyniowe doprowadzające do zmian niedokrwiennych, włóknienie śródmiąższowe, postępujące twardnienie kłębuszków czy zanik kanalików i apoptoza komórek nabłonkowych.

Słowa kluczowe: Cyklosporyna A, nefrotoksyczność, stres oksydacyjny, mitochondria