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Long-term cyclosporine treatment: Evaluation of serum biochemical parameters and histopathological alterations in Wistar rats

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ABSTRACT

The immunosuppressant agent cyclosporine (CsA) is currently used in transplanted patients and in the therapy of autoimmune disorders. CsA treatment has significant acute and chronic side effects on the liver and kidney. However, in the clinical setting, it is difficult to distinguish a direct effect of CsA treatment from other confounding variables, such as allograft rejection and effects due to other drug therapies. In the present study, we assessed for direct associations between CsA immunosuppressive therapy and cytokines levels, kidney and liver functionality, as well as lung histopathological status in rats submitted to chronic CsA treatment without undergoing any transplantation. Male Wistar rats were divided into three groups. The control group received vehicle (corn oil), and treated groups received CsA 5 or 15 mg/kg, by daily gastric gavage during 8 weeks. The results demonstrated that CsA treatment decreases blood levels of interleukins 1α (IL-1α), 1β (IL-1β) and interleukin 2 (IL-2), but does not alter interleukin 6 (IL-6) and IFN-γ levels. Serum biochemical markers of renal (creatinine) and hepatic (SGPT and SGOT) injury/dysfunction did not vary with CsA treatment, despite the presence of small histological alterations, suggesting that the function of these metabolic organs were preserved. Pulmonary histopathological lesions were observed in the CsA groups, and they were attributed to the activation of the local immunoresponse mechanisms by the normal microbiota in immunosuppressive CsA cases. These results suggest that the CsA concentrations administered in our experimental protocol were able to induce immunosuppression in rats without causing nephro and hepatotoxicity.

1. Introduction

Cyclosporine A (CsA) is widely utilized as an immunosuppressant in organ transplantation, as well as in the therapy of several immunodisorders (Borel et al., 1996). However, its clinical use is often limited by severe side effects. CsA inhibits the production of interleukins, gamma-interferon (IFN-γ) and other lymphokines (Ho et al., 1996; Zhang et al., 2005). These cytokines, which modulate the immune and inflammatory reactions, present diverse physiological roles regulating the innate and adaptative immunity, as well as stimulating the hematopoiesis. Thus, the blood levels of interleukins 1α (IL-1α) and 1β (IL-1β), interleukin 2 (IL-2), interleukin 6 (IL-6) and IFN-γ may be used as parameters for evaluating the CsA immunosuppressive effects.

CsA treatment has significant acute and chronic side effects on the liver and kidney (Grub et al., 2000a, b). *In vivo*, CsA increases lipoperoxidation in rat kidney and liver, depletes the hepatic and renal pool of glutathione (Wolf et al., 1997), and impairs antioxidant defense systems.

The histopathological changes occurring in the liver comprise sinusoidal dilatation, cytoplasmic vacuolization of hepatocytes, cell infiltration (especially in the periportal areas), parenchymal mitosis and moderate hepatocellular necrosis. The mechanisms underlying the hepatic side effects have not been explained despite extensive studies (Diao et al., 2002). Chronic CsA nephropathy is characterized by irreversible renal-striped interstitial fibrosis, inflammatory cell infiltrations and hyalinosis of the afferent glomerular arterioles (Bennett et al., 1996; Myers et al., 1984).

Since most of CsA studies have been done in transplanted recipients, the presence of multiple confounding factors in the clinical setting, such as allograft rejection and other drugs therapies, hinders the evaluation of specific effects of CsA on the

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organs functions. Thus, in the present study, we aimed to determine direct associations between CsA immunosuppressive therapy and cytokines levels, kidney and liver functionality, as well as lung histopathological status in rats submitted to a chronic CsA treatment without undergoing any transplantation.

2. Materials and methods

2.1. Animals and treatments

Rats were obtained from the Central Animal House of the Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. Adult male Wistar rats, 120 days old, weighing 300–350 g, were maintained under a standard dark–light cycle (lights on between 7:00 a.m. and 7:00 p.m.), at a room temperature of 22 ± 2 °C. The rats had free access to food (standard laboratory rat chow) and water. Animal care followed the official governmental guidelines in compliance with the Federation of Brazilian Societies for Experimental Biology and was approved by the Ethics Committee of the Federal University of Rio Grande do Sul, Brazil.

Rats were divided into three groups ($N=10$/group) according to the treatment schedule. The control group received vehicle (corn oil) and treated groups received CsA 5 mg/kg or CsA 15 mg/kg. The drug or vehicle administration was performed by a daily gastric gavage during 8 weeks. The animals were anesthetized 24 h after the last CsA or vehicle administration with sodium thiopental (40 mg/kg). Blood samples were collected by cardiac function and then animals were killed by decaptation, and organs were removed immediately.

2.2. Biochemical analysis

CsA concentration in whole blood was determined by enzyme multiplied immunoassay test (EMIT-Green Liquid, Dade-Behring on Cobas Mira, Roche Diagnostic Systems, USA). Serum IFN-γ and interleukins 1α (IL-1α), 1β (IL-1β), 2 (IL-2) and 6 (IL-6) levels were measured using commercially available ELISA kits (Quantikine, R&D Systems, USA).

Serum biochemical parameters as creatinine, glutamic oxalacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) were analyzed using commercial kits manufactured by Roche (USA) in automatized equipment (Cobas Integra 400, Roche, USA).

2.3. Morphological evaluation

Morphological data were collected from 30 treated animals. Kidney, liver and lung were removed and fixed with 10% buffered formalin at room temperature. Tissues were dehydrated in gradual alcohol from 50% to 100%, cleared by xylene and embedded in paraffin. Two to five micrometers-thick sections were stained with hematoxylin and eosin (H&E) to observe the general structure. Kidney sections were also stained with periodic acid-Schiff's reagent (PAS). A minimum of 10 fields at 100× and 400× magnifications from each slide were assessed and graded in each biopsy. Slides were evaluated by two blinded histologists under light microscopy.

Semi-quantitative scores were used to evaluate the extent of changes in tissues sections from each group (Shihab et al., 1996).

Kidney: For tubular injury the following score was used: 0=no tubular injury, 1= < 25% of tubules injured, 2=from 25% to 50% of tubules injured, 3=from 50% to 75% of tubules injured, 4=more than 75% of tubules injured. Interstitial damage was estimated semi-quantitatively using the following scores: 0=normal interstitium, 1= < 25% of areas injured, 2=from 25% to 50% of areas injured, 3=from 50% to 75% of areas injured, 4=more than 75% of areas injured. The hyalinosis, identified through the PAS staining, consisted of hyaline deposition within the tunica media of afferent arterioles and terminal portions of interlobular arteries. It was assessed in the afferent arterioles using the scores as follows: 0=no arterioles injured, 1= < 25% of arterioles injured, 2=from 25% to 50% of arterioles injured, 3=from 50% to 75% of arterioles injured, 4= > 75% of arterioles injured.

Liver: The hepatic changes examined consisted of sinusoidal dilation, cell infiltration and hepatocellular vacuolization according to Kurus et al. (2008). Sinusoidal dilatation was graded as follows: 0=normal sinusoids, 1=mild dilatation, 2=moderate dilatation and 3=severe dilatation. Cell infiltration was graded as follows: 0=normal parenchyma and portal areas, 1=mild infiltration especially in the periportal areas, 2=moderate infiltration and 3=widespread infiltration. Hepatocellular vacuolization as follows: 0=normal hepatocytes, 1=mild vacuolization, 2=moderate vacuolization and 3=severe vacuolization.

Lung: Pulmonary changes evaluation was focused on bronchiolar associated lymphoid tissue (BALT), lymphohistiocytic perivasculitis and bronchoalveolar infiltrates. BALT was graded as follows: 0=normal BALT; 1=mild BALT hyperplasia; 2=moderate BALT hyperplasia and 3=severe BALT hyperplasia. Lymphohistiocytic perivasculitis was graded as follows: 0=normal vessels; 1=mild perivasculitis; 2=moderate perivasculitis and 3=severe perivasculitis. Bronchoalveolar infiltrates was graded as follows: 0=no bronchoalveolar infiltrates; 1=mild bronchoalveolar infiltrate, 2=moderate bronchoalveolar infiltrate and 3=severe bronchoalveolar infiltrate.

2.4. Statistical analysis

Continuous data were analyzed using one-way ANOVA, followed by the Tukey's multiple range tests. $P \leq 0.05$ was considered to represent a statistically significant difference. Biochemical analyses were performed using the Statistical Package for Social Sciences (SPSS) software.

chi-square for trend was used to check the hypothesis of linear trend between the treatments and the presence of lesions. For this, the control group was used as dummy variable in order to compare the odds of lesion according to treatment protocol (i.e. 5 or 15 mg of CsA). This analysis was performed using the software EpiInfo 6.0.

3. Results

3.1. Cyclosporine serum levels

Blood CsA concentration 24 h after the last CsA administration is shown in Table 1. CsA 15 mg/kg group presented higher CsA

Table 1
Serum biochemical parameters in control and CsA-treated rats.

Variable	Control	CsA 5 mg/kg	CsA 15 mg/kg
CsA (ng/mL)	Not detectable	92.4 ± 30.1	$425.7 \pm 66.5^{**}$
IFN-gamma (pg/mL)	229.0 ± 18.0	223.0 ± 13.0	242 ± 21.7
Creatinine (mg/dL)	0.68 ± 0.05	0.66 ± 0.06	0.70 ± 0.08
SGOT (U/L)	69.0 ± 18.4	71.0 ± 20.5	58.0 ± 10.1
SGPT (U/L)	21.2 ± 5.5	23.4 ± 7.6	17.8 ± 6.7

Data are given as mean ± standard deviation. $^{**}P \leq 0.001$ compared with CsA 5 mg/kg group.