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Original article

Plant flavone apigenin protects against cyclosporine-induced histological and biochemical changes in the kidney in rats

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ABSTRACT

Cyclosporine (CsA) is an immunosuppressant drug universally used for the prevention of transplant rejection and also for the treatment of autoimmune diseases. Use of cyclosporine has been limited by its side effects such as hypertension and renal damage. Antioxidants are known to protect free radical induced damage of tissues during drug toxicity. The aim of this study was to test the role of plant flavone apigenin against cyclosporine-induced nephrotoxicity. Adult male Sprague Dawley rats were randomly divided into control, cyclosporine alone, and cyclosporine with apigenin (10, 15 and 20 mg/kg). Cyclosporine treatment was continued for 21 days to induce nephrotoxicity. From the blood samples, urea, uric acid, total antioxidants and lipid hydroperoxide assays were done. There was a significant renal damage with cyclosporine alone treatment. Blood urea nitrogen, urea, uric acid and lipid hydroperoxides were significantly elevated whereas there was a significant decrease in the total antioxidant levels. Treatment with apigenin significantly reduced the lipid hydroperoxides and increased the total antioxidant levels. Concurrent apigenin treatment significantly reduced the histopathological changes in the CsA treated groups. In conclusion, the study confirmed the role of oxidative stress in the pathogenesis of cyclosporine-induced nephrotoxicity and protective effects of flavone apigenin against free radical-induced renal damage.

1. Introduction

Cyclosporine (also known as Ciclosporin or Cyclosporin A) is a cyclic peptide of 11 amino acid residues isolated from the fungus *Tolypocladium inflatum*. Cyclosporine (CsA) is the first immunosuppressant that was found to allow selective immunoregulation of T cells without excessive toxicity [1,2]. The introduction of cyclosporine has revolutionized organ transplantation. It is found to be highly effective in the prevention of transplant rejection, while leaving the functioning of the rest of the immune system largely intact [3,4]. It is currently used to prevent graft rejection in the kidney, liver, heart, lung, combined heart-lung transplants, rejection following bone marrow transplantation, and also in the prophylaxis of host-versus-graft disease. Cyclosporine is also used in most autoimmune diseases apart from transplantation. The cyclosporine dose used for autoimmune diseases is usually lower than the dose used in transplantations and is often temporary [2,5]. Therapeutic drug monitoring is needed for the use of CsA. The therapeutic plasma concentration range is from 50–200 μg/L, and the toxic level is above 200 μg/L [6].

Cyclosporine has various unwanted side effects. The common and most important side effect is nephrotoxicity [4,7]. Hepatotoxicity and hypertension are also common complications of cyclosporine treatment. Among the various adverse effects of CsA, nephrotoxicity is the limiting factor in the use of cyclosporine in various patients [8]. This renal damage is dose-dependent and becomes increasingly common with them. The renal function may still be adversely affected even with careful adjustment of the dose in response to trough blood levels. The pathogenesis of CsA-induced nephrotoxicity is not fully understood at the moment. Studies have proposed various mechanisms that may lead to it. Studies have suggested that the generation of ROS and oxidative stress have been implicated in the pathogenesis of cyclosporine-induced nephrotoxicity [9–11].

Dietary antioxidants are thought to reduce cardiovascular diseases by reducing the formation of free radicals and oxidative stress in general, by LDL oxidation and platelet aggregation

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protection, and also by inhibiting proinflammatory cytokines synthesis [12,13]. Flavonoids are polyphenolic compounds and are subdivided into six subgroups according to their structural patterns; flavonols, flavones, isoflavones, flavonones, flavanols, and anthocyanidins. Reports have also indicated that they have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities [14,15]. 4′,5,7-Trihydroxyflavone, also known as apigenin, is a nontoxic and non-mutagenic common dietary flavonoid and is classified under the flavone group with a skeleton of 2-phenylchromen-4-one [16,17]. Apigenin can be found in many fruits, vegetables and herbs such as onions, parsley, tea, chamomile, lemon balm, perilla, apples, oranges, wheat sprouts, and also in honey and some seasonings [18,19]. Dietary flavonoid apigenin is expected to have therapeutic potential against malignant tumors. Recent studies have shown that apigenin has a free radical scavenging effect [17,20,21]. Because of its antioxidant potential and the lack of any report on its protective effect in nephrotoxicity in modern researches, this study was initiated. The aim of the study was to evaluate the potential protective effect of the flavonol apigenin in cyclosporine-induced nephrotoxicity in rats. Our research hypothesis was: apigenin will protect against cyclosporine-induced nephrotoxicity.

2. Materials and methods

2.1. Chemicals

The following chemicals were used; cyclosporine (Novartis, Malaysia), apigenin (Sigma Aldrich, USA), phosphate buffer (10 mM, Calbiochem, USA), formaldehyde (Sigma Aldrich, USA), sodium pentobarbital (Sigma Aldrich, USA). All the solvents are of analytical grade and obtained from Sigma Aldrich (USA).

2.2. Animals

The study was performed on adult male Sprague Dawley rats with the body weight range of 200–250 g. The rats were bred and kept at the animal holding facility of the International Medical University (IMU), Kuala Lumpur. The animals had free access to drinking water and rat pellets throughout the duration of the experiment. Rats were housed in a well-ventilated room under a 12/12 h light/dark cycle at 24 ± 2 °C. All experimental procedures were carried out according to the EU Directive 2010/63/EU for animal experiments and the study got the approval from the Institutional Ethics Committee.

2.3. Animal grouping

Rats were randomly divided into 5 groups ($n = 8$ in each group). Control groups of rats were maintained in the standard laboratory conditions and were treated with olive oil (vehicle of CsA) orally. Rats in the cyclosporine alone (CYS) group received cyclosporine orally (20 mg/kg body weight) through feeding needles for a duration of 21 days. The rats with apigenin treated groups received daily cyclosporine and apigenin. Apigenin (10, 15 and 20 mg/kg body weight, daily) was administered through rodent feeding needles immediately after cylcosporine treatment.

Twenty-four hours after the last treatment, on day 22 the rats were anesthetized with sodium pentobarbital (40 mg/kg body weight) and blood samples were collected by cardiac puncture. The kidneys were removed; cleaned and wet weight was noted. The blood was centrifuged at 3000 RPM in 4 °C for 15 minutes and serum was separated. The serum was stored at −20 °C for biochemical estimations.

2.4. Biochemical assays

The blood urea nitrogen, urea and uric acid levels were determined by spectrophotometric methods using the respective commercially available kits (BioAssay Systems, USA). The serum and kidney homogenate (the left kidney was perfused with ice cold saline and homogenized in chilled phosphate buffer) were used for the estimation of total antioxidants and lipid hydroperoxides using commercially available EIA kits (Cayman Chemical Company, USA).

2.5. Histological study

The right kidney was sectioned and fixed at 10% neutral buffered formalin for at least 24 hours and the tissues were processed for microscopical examination using standard protocol. Tissue sections of 4 m were stained with hematoxylin-eosin (H&E). A minimum of 10 fields for each kidney slide were examined with Nikon Microscope ECLIPSE 80i and assigned for severity of changes by an observer blinded to the treatments of the animals. The severity of the changes was determined using scores of none (−), mild (+), moderate (++) and severe (+++).

2.6. Statistical methods

Statistical analysis was carried out using one-way analysis of variance followed by Tukey's test using SPSS software (SPSS INC., USA). Data were expressed as mean ± SE. Sequential differences among means were calculated at the level of $P < 0.05$.

3. Results

A significant increase ($P < 0.05$) in the blood urea nitrogen, creatinine, urea and uric acid were seen in rats treated with CsA alone, when compared to control animals. Apigenin treatment significantly decreased ($P < 0.05$) blood urea nitrogen, creatinine, urea and uric acid levels. The levels of these parameters were significantly lower ($P < 0.05$) than the CsA alone group (Table 1).

Total antioxidant level from the kidney homogenate was significantly decreased ($P < 0.05$) in the CsA group indicating a significant oxidative stress in the kidney. Treatment with apigenin significantly increased the total antioxidant levels ($P < 0.05$). The total antioxidant elevated with all three doses of apigenin and there were no significant differences in the antioxidant levels between three groups. Lipid hydroperoxide levels in the kidney increased significantly ($P < 0.05$) with cyclosporine treatment for 21 days ($P < 0.05$). There was more than 100% increase in the lipid hydroperoxide levels in the kidney homogenates of cyclosporine treated group when compared with the control animals. Apigenin treatment along with cyclosporine was able to reduce the lipid hydroperoxides to a significant extent ($P < 0.05$), even though the levels remained higher than the control. No statistically significant difference was noted in lipid hydroperoxide levels between three doses of apigenin treatment (Table 2).

Histology of the kidney showed severe glomerular atrophy and congestions of interstitium and the blood vessel. Tubular necrosis and cast formations were seen in CsA alone treated kidneys. The non-necrosed glomerulus showed severe reduction in the size and there were hyalinization in many areas. With apigenin treatment, a significant reduction in the CsA renal damage was seen. All three doses of apigenin treatment group showed many normal glomeruli in the kidney histology. There were minimal blood vessel thickening and areas of interstitial congestion were also significantly reduced. Occasional tubular atrophy and very mild interstitial edema was seen in CsA with apigenin treated kidneys (Table 3; Fig. 1).