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Effects of antioxidant agents against cyclosporine-induced hepatotoxicity

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ABSTRACT

Background: To investigate the potential protective antioxidant role of ursodeoxycholic acid (UDCA), melatonin, and allopurinol treatment in cyclosporine (CsA)-induced hepatotoxicity.

Methods: Hepatotoxicity was established in Sprague–Dawley rats by daily administration of CsA. Treatment groups were additionally administered UDCA, melatonin, or allopurinol treatments. Rats that received no CsA and no treatments served as a control group. Liver samples from each group were examined by histopathologic analysis to determine the effects of CsA treatment on liver morphology. Biochemical assays were also used to determine the effect of CsA treatment on liver function, in the presence or absence of UDCA, melatonin, or allopurinol.

Results: CsA treatment induced hepatotoxicity, resulting in sinusoidal dilatation, congestion, infiltration, hydropic degeneration, and loss of glycogen storage in the liver. From a molecular perspective, the CsA treatment increased levels of malondialdehyde (MDA) levels, decreased levels of reduced glutathione and xanthine oxidase, and decreased activities of superoxide dismutase and catalase. The CsA treatment also resulted in decreased serum total antioxidant capacity, whereas alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin levels, and total oxidant status were increased. Treatment with UDCA, melatonin, or allopurinol reduced the CsA-induced histopathologic changes, as compared with CsA-treated samples. In addition, UDCA, melatonin, or allopurinol treatment mitigated the CsA-induced effects on glutathione and MDA levels, and on superoxide dismutase and catalase activities, as well as reduced the CsA-mediated perturbations in serum levels of total antioxidant capacity, total oxidant status, and alkaline phosphatase.

Conclusions: UDCA, allopurinol, and melatonin may each help to protect against CsA-induced damage to liver tissues, possibly through effects on the antioxidant system.

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1. Introduction

Cyclosporine (CsA), formerly referred to as cyclosporine A, is a hydrophobic cyclic polypeptide produced by the fungus *Tolypocladium inflatum*. The use of CsA as an immunosuppressant in allotransplantation has revolutionized the management of graft rejection [1,2]. CsA inhibits the T cell receptor signal transduction pathway and is most frequently used in transplant surgeries or autoimmune diseases [3]. Unfortunately, CsA treatment results in adverse side effects, including hepatotoxicity, nephrotoxicity, cardiotoxicity, and neural toxicity [2–5]. Despite extensive research, the molecular mechanism of CsA-induced toxicity remains unclear. Previous studies have demonstrated that CsA treatment results in reactive oxygen species (ROS) production and oxidative stress [2,6,7]. Oxidative stress is defined as an imbalance between the production of oxidants in an organism and the organism's ability to neutralize reactive intermediates or repair cellular damage resulting from reactive oxidants. The liver produces numerous enzymes that act to mitigate the damage caused by ROS. In addition, it is likely that antioxidants may play a beneficial role in mitigating the effects of CsA-induced hepatotoxicity [8].

Numerous antioxidative compounds have been identified; however, a role for these compounds in managing CsA-induced hepatotoxicity has not yet been defined. Ursodeoxycholic acid (UDCA) is an effective medication used to treat primary biliary liver cirrhosis and other cholestatic liver diseases in humans [9]. UDCA treatment reduces bile acid-induced cytotoxicity and reduces stimulation of hepatobiliary secretion. Treatment with UDCA additionally promotes antioxidant activity in part due to an enhancement in glutathione (GSH) levels and inhibition of liver cell apoptosis [10]. Melatonin (N-acetyl-5-methoxytryptamine) is a pineal secretory product with well-characterized hormonal activities. In addition, melatonin treatment has been reported to induce direct and indirect antioxidant effects [11,12]. Allopurinol is a potent xanthine oxidase (XO) inhibitor and is a well-established therapeutic drug used to reduce symptoms of gout and hyperuricemia. Allopurinol significantly decreases the production of ROS by scavenging hydroxyl radicals and chelating non-catalyst bound iron, which acts to inhibit free radical formation [13–15]. We tested this hypothesis using three different antioxidant agents (UDCA, melatonin, and allopurinol) to reduce CsA-induced hepatotoxicity in experimental study.

2. Material and methods

2.1. Animals

Fifty-five male Sprague–Dawley rats (11–12 wk of age, 230–300 g in weight) were obtained from the Experimental Animal Research Center of Inonu University (Malatya, Turkey). These rats were housed under regular laboratory environment conditions (21 ± 2°C, 60 ± 5% humidity, and 12:12 h light–dark cycle) with free access to standard rat chow and water. All procedures involving these rats were designed in accordance with the Guidelines for Animal Research from the National Institute of Health and were carried out with preapproval from the Ethical Committee on Animal Research at Inonu University (number 2011/A-111).

2.2. Experimental design

The rats were randomly assigned to one of five groups ($n = 11$) for 28-d regimens of sham-treatment (control) or experimental treatments. The control rats were given a saline solution (per orally [p.o.] once daily). The experimental treatments were given as follows: CsA group, Sandimmune (100 mg/50 mL CsA solution; Novartis, Basel, Switzerland) applied as 25 mg/kg/p.o. once daily starting on day 7 and continuing through day 28; CsA + UDCA group, administered CsA as above plus UDCA (Ursofalk 250 mg capsules; Ali Raif Ilaç Sanayi, Istanbul, Turkey) applied as 25 mg/kg/p.o. twice daily from day 1–day 28; CsA + M group, administered CsA as above plus melatonin (Melatonina 3 mg tablets; Aarti Drugs Ltd, Tarapur, India) applied as a 10 mg/kg/p.o. once daily from day 1–day 28; and CsA + A group, administered CsA as above plus allopurinol (Urikoliz 300 mg tablets; Sandoz, Novartis, Broomfield, CO) applied as 50 mg/kg/p.o. once daily from day–to day 28. The dose and duration of CsA and antioxidant agents were selected according to results from previous studies. On day 29, the body weight (in grams) of each rat was recorded, and the animals were sacrificed by ketamine (Ketalar; Eczacibasi Warner–Lambert, Levent, Istanbul, Turkey) anesthesia overdose. Blood samples were immediately collected from the vena cava inferior (for biochemical analysis), and liver tissues were excised. Each liver specimen was divided for histologic examination by light microscopy and enzymatic analyses to measure catalase (CAT), superoxide dismutase (SOD), GSH reductase, XO, and malondialdehyde (MDA) contents were performed.

2.3. Histopathologic evaluations

Each liver tissue sample was fixed in 10% formalin for 48 h before embedding in paraffin. Tissue sections were then cut into 5-μm slices and mounted on slides. Samples were stained with hematoxylin-eosin to visualize the general liver structure and periodic acid Schiff (PAS) to visualize glycogen deposition in hepatocytes. The prepared liver sections were evaluated by light microscopy (DFC280 equipped with the QWin Image Processing System; Leica Micros Imaging Solutions Ltd, Milton Keynes, United Kingdom). An experienced histologist who was blinded to the treatment group semi-quantitatively assessed the severity of liver damage in each sample according to the following criteria: sinusoidal dilatation, infiltration, congestion, vacuolization, and loss of glycogen deposition. For each sample, each of these observed parameters of hepatic damage was scored using a grading system with 0 representing absence of damage, 1 representing slight damage, 2 representing moderate damage, and 3 representing severe damage [12,16]. The total liver damage score was then calculated by summing the scores for each parameter; the total possible score was 15, which corresponded to the most severe level of liver damage. This average value for each group was reported as the mean histopathologic damage score (MHDS).

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