Melatonin Prevents Cyclosporine-Induced Hepatotoxicity in Rats

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Key Words

Cyclosporine A · Melatonin · Hepatotoxicity · Histopathology

Abstract

Objectives: Cyclosporine A (CsA) is a widely used immunosuppressive agent that is implicated in the formation of free oxygen radicals. Melatonin is known to be a free radical scavenger and an antioxidant agent. This study was designed to investigate the effects of melatonin on CsA-induced liver damage by histopathological examination. **Materials and Methods:** Thirty-two male rats of Sprague-Dawley origin were divided into 4 groups of 8 and treated for 28 days as follows: group 1 received daily doses of 0.1 ml/kg olive oil s.c.; group 2 received 4 mg/kg of melatonin; group 3 received 10 mg/kg CsA diluted in 0.1 ml/kg olive oil; group 4 was treated with 4 mg/kg melatonin i.p. and 10 mg/kg CsA s.c. Finally, the rats were sacrificed by terminal anesthesia, and liver tissue specimens were processed for light microscopy, stained with HE and examined under a light microscope. **Results:** Specimens of the control group showed normal liver histology, whereas group 3 showed major histopathological changes, such as cytoplasmic vacuolization, dilatation of the sinusoids, apoptosis and many mitotic figures. In group 4, the normal histology of the liver was preserved, although apoptosis, mitotic figures and cytoplasmic vacuolization were still infrequently observed. Nevertheless, there were significant differences between group 2 (melatonin) and

group 3 (CsA) and between group 3 (CsA) and group 4 (CsA + melatonin) concerning these 3 parameters (vacuolization, sinusoidal dilatation and apoptosis). **Conclusion:** The results of this study suggest that CsA-related liver toxicity in rats could be significantly reduced by melatonin administration.

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Introduction

Cyclosporine A (CsA), an immunosuppressive agent, is widely used for the treatment of autoimmune diseases and after organ transplantations [1–3]. However, its clinical and experimental use is limited by several side effects, such as nephrotoxicity, cardiotoxicity, hypertension and hepatotoxicity. Several mechanisms involved in some of the side effects have been extensively discussed and clarified; however, those mechanisms by which CsA causes hepatic injury are not fully understood [3]. Different authors have suggested that reactive oxygen species production, oxidative stress and depletion of the hepatic antioxidant system are possible mechanisms of CsA hepatotoxicity [4]. Impairments in liver architecture have been reported in rats given CsA for 2–13 weeks [5]. In our study, we observed that sinusoidal dilatation, apoptosis and mitotic figures in the parenchyma are the main histopathological changes seen in the liver tissue.

Melatonin (N-acetyl-5-methoxytryptamine) is the main indolamine produced in the pineal gland [1, 2, 6, 7]. Melatonin is known to have a number of beneficial effects in the organs and tissues, through having direct and indirect antioxidant effects and being a direct free radical scavenger [2, 6, 8–11]. Exogenously delivered melatonin readily passes through the morphologic barriers and prevents oxidative damage in the tissue [8].

The aim of this study was to investigate the effects of melatonin in CsA-related liver damage.

Materials and Methods

Thirty-two male Sprague-Dawley rats (Inonu University Animal Research Center, Malatya, Turkey), weighing between 230 and 300 g, were housed in individual cages for 28 days in a well-ventilated room with a 12:12-hour light/dark cycle and were fed at libitum with rat chow having a low sodium content (0.05% Na, Aytakinler, Konya, Turkey). The experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals.

Rats were randomly divided into 4 groups of 8, and were treated as follows: group 1, the control group, received 0.1 ml/kg s.c. olive oil daily; group 2 was treated with intraperitoneal daily doses of 4 mg/kg melatonin; group 3 received a daily dose of 10 mg/kg CsA s.c. (Sandimmun 50 mg/ml, Novartis, Basel, Switzerland), delivered by dilution in 0.1 ml/kg of olive oil; group 4 was given daily doses of 4 mg/kg melatonin i.p. and 10 mg/kg CsA s.c.

At the end of day 28 of the experiment, the rats were sacrificed by terminal anesthesia and tissue specimens obtained from the liver were immersed in Bouin's fixative solution. The tissues were further processed for embedding in paraffin, and 4- to 5- μ m thick sections were stained with HE and examined under a light microscope (Olympus BH2, Tokyo, Japan). Two double-blinded histologists (M.K., M.E.) assessed the cross-sections. Twenty-five lobules of liver for each rat, in total 200 lobules of liver for each group, were examined by the histologists, and scored for presence or absence of visible lesions. Vacuolization, sinusoidal dilatation, apoptosis and necrosis in all areas were identified and graded as follows: normal hepatocytes = 0, mild = 1, moderate = 2; severe = 3. The same scale was used for grading each specimen.

All parameters were expressed as means $\pm$ SD. All groups were compared by the nonparametric Mann-Whitney U test. Exact p values were given where available, and $p < 0.05$ was accepted as statistically significant.

Results

In group 1, the control group, typical liver lobules were observed with the central vein localized in the middle and hepatocyte cords radiating towards the periphery. The portal areas containing the hepatic artery, the portal vein and the bile duct were surrounded by connective tis-

Fig. 1. Liver section from group 2 showing normal hepatocytes. HE. $\times 40$.

sue. Sinusoids were observed between the hepatocyte cords, and the polygonal-shaped hepatocytes mostly contained a single oval-shaped euchromatic nucleus.

In group 2 (melatonin), there was no visible change in the normal liver histology. A typical lobular structure was observed with parenchymal cells radiating between the sinusoids. Integrity of the sinusoids was preserved, and the dimensions of the sinusoids and perisinusoidal spaces were within normal ranges. Hepatocytes demonstrated the usual polygonal shape, and their cytoplasm enclosed mostly a single oval-shaped nucleus with a single nucleolus (fig. 1).

In group 3 (CsA), major histopathological changes, such as vacuolization, dilatation of the sinusoids and apoptosis, were seen. Numerous mitotic figures, apoptotic and picnotic bodies were observed in the parenchyma. Acidophilic particles were detected in the cytoplasm of most of the cells, and increased vacuolization was observed in the cytoplasm (fig. 2).

In group 4 (CsA + melatonin), although the liver tissue preserved its normal appearance, occasional areas showed apoptosis, mitotic figures and cytoplasmic vacuolization (fig. 3). The numerical scores for vacuolization, sinusoidal dilation and apoptosis in group 3 were 1.75 ± 0.1 , 1.87 ± 0.1 and 1.25 ± 0.2 , respectively, indicating mild to moderate histopathological changes; while the numerical scores in group 4 were: vacuolization 0.25 ± 0.1 , sinusoidal dilation 0.37 ± 0.1 and apoptosis 0.25 ± 0.1 , indicating normal to mild changes.

Fig. 2. Liver sections from group 3. **a** Diffuse vacuolization (arrows). HE. $\times 100$. **b** Sinusoidal dilatation. HE. $\times 40$. **c** Apoptotic body (arrow). HE. $\times 100$. **d** Apoptotic body (arrow). HE. $\times 100$.

The differences between the control and CsA groups for vacuolization ($p = 0.000$), sinusoidal dilatation ($p = 0.000$) and apoptosis ($p = 0.002$) were statistically significant. There were also significant differences between group 2 (melatonin) and group 3 (CsA), and group 3 (CsA) and group 4 (CsA + melatonin), for same parameters ($p < 0.05$).

Discussion

It has previously been demonstrated that CsA produces oxidative stress in the primary cell cultures of rat hepatocytes, which could be inhibited by antioxidant agents [1, 12]. Hepatocytes incubated with CsA showed lipid and glycogen vacuoles during examinations at 4 and 22 h. These findings were considerably augmented at 22 h, and it was suggested that they might be due to the increased oxidative stress [1]. In conditions of stress, the first-order response is the swelling of the cell. Impairments in the energy metabolism balance and the subsequent hindering of energy related to pump systems causes a fluid and electrolyte imbalance leading to the accumulation of excessive fluid that is microscopically visible as vacuoliza-

Fig. 3. Liver section from group 4 showing normal hepatocytes (arrows). HE. $\times 40$.

tion within the cytoplasm [13], as observed in group 3 and to some extent in group 4.

Apoptosis is the result of a complex process that develops due to the disintegration of the cell membrane. This metabolic self destruction is followed by a reduction in the cell size, shrinkage of the cell, cytoplasmic vacuoliza-

tion, fragmentation of the nucleus, and condensation and clustering of chromatin. As a consequence, the cell breaks apart, forming the apoptotic bodies [13] similar to those in group 3. Recent data suggest that oxidative stress is the underlying mechanism of CsA-related apoptosis [1, 14–17].

Melatonin, the chief indolamine secreted from the pineal gland, is an effective antioxidant agent [2, 4, 18]. Antioxidant agents operate through neutralizing the toxic reactants, and reducing the molecular damage to the free radicals [2, 19]. Another activity of melatonin is to stimulate the antioxidant enzymes [7, 8, 11, 20]. Melatonin also has protective effects upon the oxidative damage developing secondary to carcinogens [6]. Carcinogenic agents, such as CsA, have been proven to produce oxidative damage by increasing the toxic free radicals [9]. Mitochondrial damage disrupts the production of ATP and leads to cell death by influencing the processes leading to apoptosis and necrosis [11, 21]. In cases of oxidative damage, melatonin can provide additional strength to the cells [8]. Treatment with melatonin recovers the hepatic functions

and prevents the liver damage triggered by free radicals [2]. In our study, normal liver histology was preserved in the melatonin-treated group (group 2). These findings show that the use of melatonin protected the normal liver histology.

It has been shown that natural antioxidants provide protective effects against CsA-induced hepatotoxicity [4]. It is possible that melatonin, a potent scavenger of radicals, could prevent or ameliorate CsA-induced hepatic injuries. This is a possible explanation for the limited histopathological changes observed in the group treated with melatonin + CsA.

Conclusions

These findings suggest that the damage caused by CsA is preventable and could be reduced by melatonin treatment. Further studies have to be carried out in order to document the effects of melatonin during long-term use of CsA.

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