



The Protective Effect of Melatonin and Agomelatin against Cisplatin-Induced Nephrotoxicity and Oxidative Stress in the Rat Kidney

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SUMMARY. Cisplatin is used to treat various types of cancers. Its use is limited, however, due to nephrotoxicity, which may result from free radical damage. Evidence exists that melatonin reduces oxidative stress-induced damage. This study investigated the protective effect of agomelatin, a melatonin receptor agonist, against cisplatin-induced nephrotoxicity and oxidative stress in the rat kidney. Groups of rats were given cisplatin with or without agomelatin or melatonin, or distilled water for 14 days. MDA, tGSH, MPO and 8-OH Gua levels were measured to determine oxidative and DNA damage in renal tissue. Levels of MDA, MPO and 8-OH Gua were lower in the Mel+Cis and Ago+Cis groups than in the Cis group ($P < 0.001$, $P < 0.001$, and $P < 0.05$, respectively). The tGSH level in the Mel+Cis group was higher than that in the Cis group ($P < 0.001$). Agomelatin and melatonin thus reduced cisplatin-induced oxidative damage and DNA damage in the rat kidney. This suggests that melatonin may be effective in preventing cisplatin nephrotoxicity.

INTRODUCTION

Cisplatin is a chemotherapeutic agent containing platinum that has long been used in the treatment of various types of cancer¹. The observed side-effects vary, depending on use, and include nephrotoxicity, ototoxicity, hepatotoxicity, myelosuppression, and spermiotoxicity. However, the most important and dose-limiting side-effect is nephrotoxicity². Cisplatin accumulates in the epithelial cells of the proximal tubules and causes severe kidney damage, attributed to oxidative stress³⁻⁵. Hydration, hypertonic saline, diuretics, antioxidant substances such as vitamin E, N-acetylcysteine and melatonin are used in order to counter cisplatin-induced nephrotoxicity⁶⁻⁹. However, nephrotoxicity cannot be prevented in 25-35% of patients receiving cisplatin.

Agomelatin is an MT1 and MT2 melatonin receptor agonist, employed in this study as a counteracting reagent to cisplatin. Due to its

anxiolytic, anti-bipolar disorder and antidepressant effects, agomelatin has been used in the treatment of sleep disorders and jet lag syndrome. It came to the fore with the discovery of its antagonist activity of serotonin 5HT_{2C} receptor^{10,11}. Previous studies have shown that some serotonergic antagonists and melatonergic agonists reduce the oxidative stress-induced toxicity of cisplatin on the kidney. Stimulation of 5-HT₂ and 5-HT₃ receptors is known to be associated with toxic side-effects¹². Mirtazapine, a 5-HT₂ and 5-HT₃ receptor blocker, is also believed to inhibit cisplatin-induced oxidative damage to the kidney¹³. Cisplatin has been reported to cause nephrotoxicity in kidney tissue by increasing the production of free radicals and reducing antioxidant production^{14,15}. Therefore, antioxidant therapy may be useful in the prevention or reduction of cisplatin-related nephrotoxicity. Information in the literature points to the protective effect of agomelatin against renal damage in

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rats. The purpose of this study was therefore to investigate the protective effects of agomelatin against cisplatin-induced oxidative stress in comparison to those of melatonin.

MATERIALS AND METHODS

Animals

Twenty-four male Sprague-Dawley rats, obtained from the Medical Experimental Practice and Research Center of Ataturk University, Turkey, and weighing 270-320 g were used. The animals were accommodated in suitable conditions (at 22 °C and fed *ad libitum*). The study was conducted in accordance with the principles of the National Institute of Health Guide for the Care and Use of Laboratory Animals, and permission was received from the local ethics committee of the Ataturk University Faculty of Medicine.

Chemicals

Cisplatin was purchased from Kocak Farma (Turkey). Melatonin was provided by Interpharm (Turkey) and agomelatin by Servier (Turkey).

Experimental design

Four groups of animals of six members each were used in the study. Group 1: agomelatin + cisplatin (Ago+Cis), Group 2: melatonin + cisplatin (Mel+Cis), Group 3: cisplatin (Cis), and Group 4: control. Groups 1, 2 and 3 were administered 2 mg/kg of cisplatin intraperitoneally (IP) in order to induce nephrotoxicity. Groups 1 and 2 received, respectively, 25 mg/kg of agomelatin and 25 mg/kg of melatonin orally via esophago-gastric tube 1 h before the application of cisplatin for 14 days. Group 3 was given distilled water (1 mL) orally via esophago-gastric tube 1 h before the administration of cisplatin for 14 days. Group 4 was given 1 mL of distilled water IP after the oral administration of 1 mL of distilled water via esophago-gastric tube for 14 days. At the end of the experimental period, the animals were anesthetized with 25 mg/kg of pentothal IP. The animals were sacrificed by cervical dislocation after removal of the kidneys for biochemical investigations. The kidneys were biochemically evaluated and the results analyzed statistically.

Biochemical analysis

Determination of malondialdehyde (MDA) formation

Concentrations of tissue lipid peroxidation were determined by estimation of MDA content

using the thiobarbituric acid test¹⁶. Briefly, the rat kidneys were promptly excised and rinsed with cold saline. To minimize any interference of hemoglobin with free radicals, tissue-adherent blood was carefully removed. The tissue was weighed and homogenized in 10 mL of KCl (100 g/L). The homogenate (0.5 mL) was added to a solution containing 0.2 mL sodium lauryl sulfate (80 g/L), 1.5 mL acetic acid (200 g/L), 1.5 mL 2-thiobarbiturate (8 g/L), and 0.3 mL distilled water. The mixture was incubated at 98 °C for 1 h. Upon cooling, 5 mL of n-butanol:pyridine (15:1) was added. The mixture was vortexed for 1 min and centrifuged for 30 min at 4000 rpm. The absorbance of the supernatants was measured at 532 nm.

Total glutathione (tGSH) determination

The amount of GSH in the tissue was measured according to the method described by Sedlak & Lindsay¹⁷. The tissue surface of the kidney was collected, weighed, and then homogenized in 2 mL of 50 mM Tris-HCl buffer containing 20 mM EDTA and 0.2 mM sucrose, pH 7.5. The homogenate was immediately precipitated with 0.1 mL of 25% trichloroacetic acid, and the precipitate was removed by centrifugation at 4200 rpm for 40 min at 4 °C. The supernatants were used to determine GSH content using 5,5'-dithiobis-2-nitrobenzoic acid. Absorbance was measured at 412 nm using a spectrophotometer.

Determination of Myeloperoxidase (MPO) activity

MPO activity was measured according to the modified method described by Bradley *et al.*¹⁸. The homogenized samples were frozen and centrifuged at 1500 g for 10 min at 4 °C. MPO activity in the supernatants was determined by adding 100 µL of the supernatant to 1.9 mL of 10 mmol/L phosphate buffer (pH 6.0) and 1 mL of 1.5 mmol/L O-dianisidine hydrochloride containing 0.0005% (wt/vol) hydrogen peroxide. The changes in absorbance at 450 nm of each sample were recorded on a UV spectrophotometer.

Isolation of DNA from Kidney Tissue

Kidney tissue was drawn and DNA isolated using the modified method described by Shigenaga *et al.*¹⁹. Briefly, 50 mg of kidney tissue was homogenized at 4 °C in 1 mL of homogenization buffer (0.1 M NaCl, 30 mM Tris, pH 8.0, 10 mM EDTA, 10 mM 2-mercaptoethanol, 0.5% (v/v) Triton X-100) with 6 passes of a Teflon-glass homogenizer at 200 rpm. The samples were centrifuged at 4 °C for 10 min at 1000 g to pellet nuclei. The supernatants were discarded,

and the crude nuclear pellets were suspended and re-homogenized in 1 mL of extraction buffer (0.1 M Tris, pH 8.0, 0.1 M NaCl, 20 mM EDTA). After centrifugation for 2 min as described previously, the washed pellets were re-suspended in 300 μ L of extraction buffer with a wide-orifice 200- μ L Pipetman tip. The solutions were subsequently incubated at 65 °C for 1 h in the presence of 0.1 mL of 10% SDS, 40 μ L proteinase K, and 1.9-mL of leukocyte lysis buffer. Ammonium acetate was then added to the crude DNA sample to give a final concentration of 2.5 mol/L, and was centrifuged in a microfuge for 5 min. The supernatants were removed and mixed with two volumes of ethanol to precipitate the DNA fraction. After centrifugation, the pellet was dried under reduced pressure and dissolved in sterile water. The absorbance of this fraction was measured at 260 and 280 nm. The purity of the DNA was determined from the A 260/280 ratio.

DNA hydrolysis with formic acid

Approximately 50 mg of DNA was hydrolyzed with 0.5 mL of formic acid (60%, v/v) for 45 min at 150 °C²⁰. The tubes were allowed to cool to room temperature. The contents were then transferred to Pierce micro-vials, covered with Kleenex tissues cut to size (secured in place using a rubber band), and cooled in liquid nitrogen. Formic acid was then removed by freeze-drying. Before analysis by HPLC, they were re-dissolved in the eluent (final volume 200 μ L).

Measurement of 8-hydroxy-2 deoxyguanine (8-OH Gua) with HPLC

Amounts of 8-OH Gua and guanine (Gua) were measured by HPLC equipped with an electrochemical detector (HP Agilent 1100 module series, E.C.D. HP 1049 A), as described previously^{20,21}. The amounts of 8-OH Gua and Gua were analyzed on a 250 x 4.6 mm Supelco LC-18-S reverse-phase column. The mobile phase

was 50 mM potassium phosphate, pH 5.5, with acetonitrile (97:3 acetonitrile-potassium phosphate, v/v), with a flow rate of 1.0 mL/min. The detector potential was set at 0.80 V for measuring the oxidized base. Gua and 8-OH Gua (25 pmol) were used as standards. 8-OH Gua levels were expressed as the number of 8-OH Gua molecules/10⁵Gua molecules²².

Statistical Analysis

All data were analyzed using one-way ANOVA on SPSS 18.0 software. Differences among groups were determined using the Tukey multiple comparison test, and significance was set at $P < 0.05$. Results are means \pm standard error of the mean (SEM).

RESULTS

As shown in Table 1, the MDA level in the Cis group was higher than that in the control group ($P < 0.001$). Conversely, it was lower in the Ago + Cis and Mel + Cis groups than in the Cis group ($P < 0.001$) (Fig. 1). tGSH level in the Cis group was lower than that of the control group ($P < 0.001$), whereas it was higher in the Mel + Cis group than the Cis group ($P < 0.001$). The tGSH level in the Ago + Cis group was slightly elevated, but this was not statistically significant ($P > 0.05$) (Fig. 2). MPO activity in the Cis group was higher compared with that in the control group, but lower in the Ago + Cis and Mel + Cis groups than in the Cis group ($P < 0.001$) (Fig. 3). While 8OH-Gua levels in the Cis group were higher than in the control group ($P < 0.05$), they were lower in the Ago + Cis ($P < 0.05$) and Mel + Cis groups ($P < 0.01$) than in the Cis group (Fig. 4).

DISCUSSION

This study investigated the protective effects of agomelatin against cisplatin-induced oxidative stress in rat kidneys and evaluated these in comparison to melatonin. The experimental results indicate that cisplatin induces oxidative

Groups	N	MDA	P*	GSH	P*	MPO	P*	8-OH Gua	P*
Ago+Cis	6	23.2 \pm 2.7	0.001	13.8 \pm 0.6	0.497	25.2 \pm 2.9	0.001	1.4 \pm 0.4	0.092
Mel+Cis	6	9.5 \pm 1.1	0.001	39.3 \pm 3.8	0.001	10.3 \pm 1.9	0.001	0.9 \pm 0.1	0.008
Cisplatin	6	46.5 \pm 1.8	-	7.7 \pm 1.1	-	46.2 \pm 2.2	-	2.5 \pm 0.4	-
Control	6	7.5 \pm 0.8	0.001	47.5 \pm 4.6	0.001	8.2 \pm 0.9	0.001	1.0 \pm 0.2	0.011

Table 1. Comparisons of groups in terms of oxidant and antioxidant parameters. Ago+Cis: agomelatin + cisplatin group; Mel+Cis: melatonin + cisplatin group; MDA: malondialdehyde; GSH: total glutathione; MPO: myeloperoxidase; N: number of animals. The activities of MPO levels of GSH, MDA and 8-OH Gua in each group versus the cisplatin group were compared using one-way analysis of variance. All values are expressed as mean \pm SEM. * $P < 0.001$ was significant.

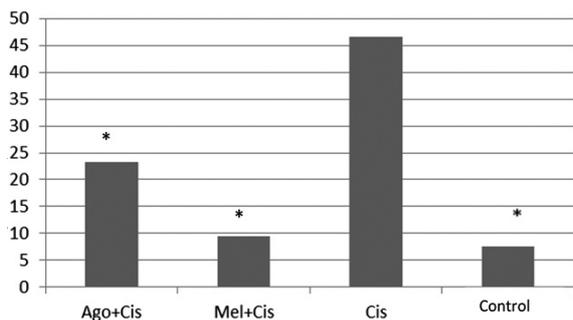


Figure 1. Comparison of groups in terms of malondialdehyde (MDA). Ago+Cis: agomelatin + cisplatin group; Mel+Cis: melatonin + cisplatin group; Cis: cisplatin group. * P < 0.001 was significant.

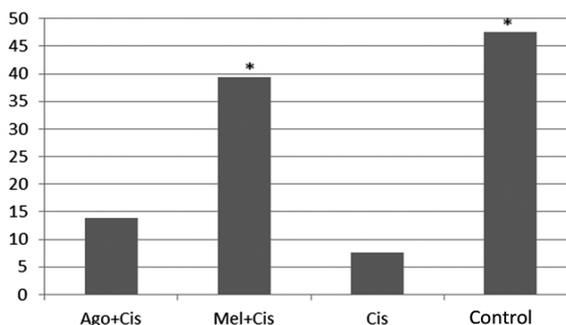


Figure 2. Comparison of groups in terms of total glutathione (tGSH). Ago+Cis: agomelatin + cisplatin group; Mel+Cis: melatonin + cisplatin group; Cis: cisplatin groups. * P < 0.001 was significant.

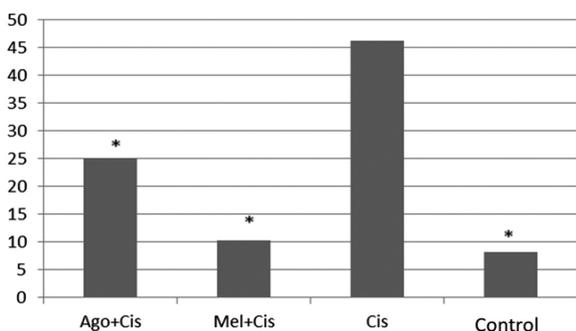


Figure 3. Comparison of groups in terms of myeloperoxidase (MPO). Ago+Cis: agomelatin + cisplatin group; Mel+Cis: melatonin + cisplatin group; Cis: cisplatin group. * P < 0.001 was significant.

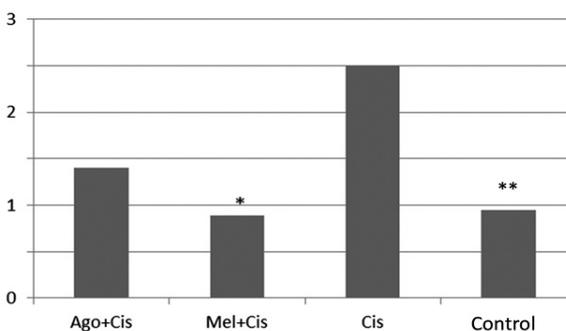


Figure 4. Comparison of groups in terms of DNA damage products. Ago+Cis: agomelatin + cisplatin group; Mel+Cis: melatonin + cisplatin group; Cis: cisplatin group. * P < 0.01. ** P < 0.05

damage in kidney tissues. The oxidant/antioxidant balance is maintained in favor of antioxidants in physiological conditions ²³. Oxidative damage occurs with the deterioration of the oxidant/antioxidant balance in favor of oxidants. Therefore, assessment of the oxidant/antioxidant balance gives an indication of tissue damage ²⁴. In oxidative damage models in live tissues, antioxidant levels have been observed to decrease and oxidant levels to increase ²⁵⁻²⁷. In this study, agomelatin significantly inhibited MPO activity and thus reduced MDA levels, which is an indicator of lipid peroxidation enhanced by cisplatin. However, it could not prevent a decrease in levels of GSH, an important intracellular antioxidant. These results show that change in the oxidant/antioxidant balance in favor of antioxidants occurs in kidney tissue treated with agomelatin and cisplatin. However, it does not prevent a decrease in GSH levels. Melatonin changed both the oxidant/antioxidant balance in favor of oxidants and prevented the disruption of the oxidant/antioxidant balance in favor of antioxidants. Agomelatin, a melatonin receptor agonist, inhibited cisplatin-induced ox-

idative renal injury, although its effect was less than that of melatonin.

Melatonin has been reported to possess radical scavenger activity, detoxifying free radicals that can cause oxidative stress ²⁸, to terminate lipid peroxidation by capturing peroxy radicals ²⁹, and to raise GSH levels by stimulating g-glutamylcysteine synthetase ³⁰. Melatonin can reach the cell nucleus and thus provides protection against oxidative damage to DNA ³¹. Insufficient antioxidant defense mechanisms lead not only to lipid peroxidation, but also to oxidative DNA damage ³². DNA damage caused by OH⁻ and ¹O₂ radicals leads to the formation of mutagenic products, such as 8-OH Gua ³³. OH⁻ radicals react with the C4, C5, and C8 positions of guanine and generate DNA adducts. The C8-OH adduct, formed by the binding of the OH⁻ radical to C-8, loses an electron and a proton and is oxidized to 8-OH Gua ³⁴. The amount of 8-OH Gua in the kidney tissue in the Ago + Cis group was higher than that in the Mel + Cis and control groups. The presence of a higher amount of 8-OH Gua in the Ago + Cis group may be due to insufficient GSH induced by agomelatin. In a

previous study, the amount of 8-OH Gua was reported to be higher in damaged tissues with a low amount of GSH³⁵. Cisplatin has been reported to reduce the amount of GSH and increase the level of 8-OH Gua in rat ovaries³⁶. Another study showed that elevated concentrations of MDA, MPO and 8-OH Gua were correlated with low levels of GSH³⁷. Although there are studies in the literature confirming the prevention of cisplatin-induced kidney damage by melatonin, similar studies on agomelatin are lacking³⁸. Agomelatin, unlike mirtazapine, lacks antioxidant properties even though it is a serotonin receptor antagonist. Furthermore, agomelatin does not prevent oxidative damage, as melatonin does, but induces it.

Consequently, potent antioxidant activity occurs as a result of the stimulation of the MT1 and MT2 receptors. We conclude that serotonin 5-HT2 receptor blockade is not significant in the prevention of oxidative stress. However, before deciding whether these receptors are responsible for the antioxidant activity, the effects of agomelatin on the MT1, MT2 and 5-HT2 receptors need to be investigated.

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