The Protective Effect of Chrysin Against Cisplatin Induced Ototoxicity in Rats

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Abstract Ototoxicity is a common side effect of cisplatin chemotherapy. The aim of this study was to investigate the potential protective effect of chrysin against cisplatin-induced ototoxicity. Thirty-four adult female Wistar albino rats were separated into four groups: a cisplatin group (Group A), with cisplatin administered to ten rats once daily for three consecutive days at doses of 8 mg/kg body weight intraperitoneally (i.p.); a cisplatin plus chrysin group (Group B), with 8 mg/kg of cisplatin administered i.p. daily to ten rats for three consecutive days and 25 mg/kg of chrysin administered via oral gavage in a corn oil for 5 days; a chrysin group (Group C), with 25 mg/kg of chrysin administered via oral gavage in corn oil for 5 days to seven rats; and a control group (Group D), with 5 ml/kg of corn oil administered to seven rats via oral gavage for 5 days. Distortion product otoacoustic emission measurements were performed in the same ear of the rats under general anesthesia at baseline and on the first and fifth days after drug administration. No significant differences were noted between the measurements either in the chrysin group or in the control group. In the cisplatin group, there

was a significant worsening of hearing compared to baseline and the measurements on the fifth day at all frequencies. In the statistical analysis, a statistically significant difference was observed at 5039, 6351, 8003, and 10078 Hz frequencies between the measurements on the first and fifth days. In the cisplatin plus chrysin group, there were statistically significant differences at frequencies of 2,003 and 5,039 Hz between the measurements at baseline and on the fifth day, at 3,175 and 5,039 Hz between the measurements on the first and fifth days, and at 8,003 and 100,078 Hz between the measurements at baseline and on the first day. According to these results, this study demonstrates that cisplatin-related ototoxicity can be prevented in rats by the administration of chrysin.

Keywords Ototoxicity · Cisplatin · Chrysin · Otoacoustic emissions

Introduction

Cisplatin is a therapeutic agent that is widely used for a number of human cancers, including head and neck, testis, ovary, lung, and bladder carcinomas [1]. Ototoxicity, neurotoxicity, and nephrotoxicity are dose-limiting side effects of cisplatin treatment. Ototoxicity may occur within hours to days after cisplatin administration [1]. Cisplatin-induced ototoxicity is frequently bilateral and progressive and results in irreversible sensorineural hearing loss, leading to decreased quality of life in cancer patients [2].

The precise mechanisms of cisplatin-induced ototoxicity are not fully understood, but excessive production of reactive oxygen species (ROS) in cochlear tissues is thought to be the likely mechanism [3]. Cisplatin administration also decreases antioxidant enzymes in the cochlea

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[4]. When the balance between the production of ROS and antioxidative defense is impaired, oxidative stress can occur, which can lead to cochlear cell injury or death.

Amelioration of cisplatin-induced ototoxicity has been demonstrated in studies, with many reporting on the protective effect of antioxidant agents, such as caffeic acid phenethyl ester [5], D-methionine [6], dexamethasone [7], neurotrophins [8], flunarizine [9], amifostine [10], *N*-acetyl cysteine [11, 12], erdosteine [13], salicylates [14], ginkgo biloba extract [15], allopurinol, ebselen [16], resveratrol [4, 17], thymoquinone [18], betaglucon [19], and lycopene [20].

Chrysin (5,7 dihydroxy-flavone) is a natural flavonoid contained in many plant extracts, honey, and propolis [21, 22]. Chrysin exhibits many biological activities and pharmacological effects, including antioxidant [23], anti-inflammatory [24], antiallergic [25], anticancer [26], anxiolytic [27], and antiestrogenic [28] properties.

There are some studies on the protective effects of chrysin on the kidney and colon during cisplatin therapies. However, there are no previously published reports regarding the preventive effect of chrysin on cisplatin-induced ototoxicity. Therefore, the present study was planned to investigate the efficacy of chrysin in preventing cisplatin-related ototoxicity.

Materials and Methods

This study was approved by the University Experimental Animal Ethics Committee. Thirty-four female Wistar albino 3-month-old rats weighing 200–260 g were used for the present study. The rats were maintained under a light–dark photo period of 12:12 h at 21 °C. The animals were fed with a standard commercial diet and given water ad libitum before and during the experiment.

To evaluate their hearing, the animals were given anesthesia intramuscularly, using 40 mg/kg of ketamine and 5 mg/kg of xylazine. After the external ear canals and tympanic membranes of the animals were examined with otomicroscopy, distortion product otoacoustic emission (DPOAE) measurements were carried out in a quiet environment. The DPOAEs were taken from the right ear of the control and the experimental rats using a standard commercial GSI Audera DPOAE (Grason Stadler, Madison, USA) device. An earphone with a plastic adapter was inserted in the animal's outer ear canal. The rats with normal DPOAE measurement results before administration of any substance on day zero and normal otoscopic findings were included in the study.

The 34 rats were randomly divided into four groups. In Group A, ten rats received 8 mg/kg of cisplatin intraperitoneally (i.p.) once daily for three consecutive days; in

Group B, ten rats received 8 mg/kg of cisplatin i.p. once daily for three consecutive days plus 25 mg/kg of chrysin via oral gavage once daily every day between day zero and the fifth day; in Group C, seven rats received 25 mg/kg of chrysin via oral gavage once daily every day between day zero and the fifth day; in Group D, seven rats received 5 ml/kg of corn oil (vehicle for chrysin) via oral gavage every day between the zero day and the fifth day.

The DPOAE measurements were taken with a GSI Audera DPOAE (Grason Stadler, Madison, USA) device before administering the drug and at 1 and 5 days after administration of the drug. The placement and the calibration of the probe were made automatically by the measuring system before the test. For the DP-gram measurements, primer stimulus levels were equalized at 65 dB ($L_1 = L_2$). Two different frequencies (f_1 and f_2) were organized as $f_2/f_1 = 1.22$ that the most powerful responses may have been taken. The measurements were performed at frequencies of 2003, 2519, 30175, 5039, 6351, 8003, and 10078 Hz, and the results were recorded. The resolutions of the DP-gram were obtained at four points per octave. The noise floor level was measured by similar techniques at a frequency of 50 Hz above the DPOAE frequency. The response was accepted as emitted when the DPOAEs were >3 dB above the noise floor level at a frequency of $2f_1 - f_2 + 50$ Hz.

The results were analyzed statistically with the Shapiro–Wilk normality test to determine the differences in the DPOAE amplitudes and the corresponding noise floor differences for each frequency. The Wilcoxon test was used for evaluating intragroup changes. $P < 0.05$ was considered statistically significant. The data were analyzed with SPSS for Windows 17.0.

Results

The DP-gram results of all the groups before and after the administration of the drug are presented in Fig. 1 and Table 1.

In Group A, two of the ten rats did not wake up after anesthesia during the measurement on the first day, and two rats died after deterioration of the health in the period between the measurements made on days one to five. The six rats that survived were evaluated. In Group A, there was a statistically significant worsening of hearing compared to the measurements at baseline and at 5 days at all frequencies ($P < 0.05$) and at frequencies of 5039, 6351, 8003 and 10078 Hz between days one and five ($P < 0.05$). No statistically significant difference was observed between day zero and the first day ($P > 0.05$).

In Group B, one of the ten rats did not wake up after anesthesia during the measurement on the first day, and two

Fig. 1 **a** DP-gram results of cisplatin group (Group A); there are statistically significant differences between baseline and fifth day measurements at all frequencies and at frequencies of 5039, 6351, 8003 and 10078 Hz between days 1th and 5th ($P < 0.05$). **b** DP-gram results of cisplatin + chrysin group (Group B); there are statistically significant differences between baseline and first day measurements at frequencies of 8,003 and 10,078 Hz, between baseline and fifth day

measurements at frequencies of 2,003 and 5,039 Hz, between 1th and fifth day measurements at frequencies of 8,003 and 10,078 Hz ($P < 0.05$). **c** DP-gram results of chrysin group (Group C); there are no statistically significant differences on the DP-gram measurements ($P > 0.05$). **d** DP-gram results of chrysin solvent (Control) group (Group D); there are no statistically significant differences on the DP-gram measurements ($P > 0.05$)

Table 1 Standard error of mean (mean $\pm$ SEM) values of; cisplatin only used Group (A), cisplatin plus chrysin used Group (B), chrysin only used Group (C), control group (D)

	2003 Hz	2,519 Hz	3,175 Hz	3,996 Hz	5,039 Hz	6,351 Hz	8,003 Hz	10,008 Hz
A								
0 day	1.9 $\pm$ 5.9	5.5 $\pm$ 5.4	11.1 $\pm$ 8.78	7.6 $\pm$ 9.3	12.5 $\pm$ 8.7	18 $\pm$ 7.2	26.5 $\pm$ 0.704	31.5 $\pm$ 5.2
1st day	-0.1 $\pm$ 2.6	3.2 $\pm$ 4.4	9.8 $\pm$ 0.5.1	4 $\pm$ 6.8	12.2 $\pm$ 5.3	1.3 $\pm$ 5.6	25.8 $\pm$ 6.6	31.2 $\pm$ 5.1
5th day	-1.5 $\pm$ 3.2*	-1.3 $\pm$ 0.4.5*	1.1 $\pm$ 6.5*	-3.9 $\pm$ 0.6.9*	-1.7 $\pm$ 6.2*	4.4 $\pm$ 5.5*	14.2 $\pm$ 6.9*	16.7 $\pm$ 4.6*
B								
0 day	3.82 $\pm$ 5.4	6.7 $\pm$ 3.2	13.1 $\pm$ 4.6	15.6 $\pm$ 6.1	22.5 $\pm$ 6.5	23.2 $\pm$ 4.5	30.7 $\pm$ 0.3.8	36.9 $\pm$ 2.6
1st day	2.6 $\pm$ 5.8	6.5 $\pm$ 8.6	13.9 $\pm$ 0.9.6	15.5 $\pm$ 9.1	24.4 $\pm$ 8.9	24.4 $\pm$ 5.5	25.4 $\pm$ 2.9*	34.1 $\pm$ 1*
5th day	-0.56 $\pm$ 4.8*	3.04 $\pm$ 0.7.6	6.8 $\pm$ 10.4*	11.7 $\pm$ 8.6	13.9 $\pm$ 12.6*	15.8 $\pm$ 5.5	23.6 $\pm$ 6.4	37.2 $\pm$ 2.6
C								
0 day	1.6 $\pm$ 2.7	6.5 $\pm$ 6.4	13.8 $\pm$ 6.7	14.8 $\pm$ 6.04	24.8 $\pm$ 8.1	26.2 $\pm$ 7.6	28.4 $\pm$ 0.7.6	41.4 $\pm$ 4.5
1st day	0.8 $\pm$ 2.6	4.2 $\pm$ 2.5	11.2 $\pm$ 0.7.1	11.8 $\pm$ 5.5	22.2 $\pm$ 5.2	22.6 $\pm$ 4.6	30.3 $\pm$ 3.3	42.2 $\pm$ 3.05
5th day	2.08 $\pm$ 1.5	5.1 $\pm$ 0.5.1	13.8 $\pm$ 5.08	14.02 $\pm$ 3.8	23.5 $\pm$ 5.4	25.2 $\pm$ 3.7	29.2 $\pm$ 4.8	43.3 $\pm$ 3.3
D								
0 day	4.8 $\pm$ 3.1	15.6 $\pm$ 0.9	20.9 $\pm$ 2.07	17.9 $\pm$ 2.8	27.1 $\pm$ 2.5	26.5 $\pm$ 2.9	30.06 $\pm$ 1.89	40.9 $\pm$ 2.9
1st day	2.04 $\pm$ 1.1	10.6 $\pm$ 3.1	14.5 $\pm$ 6.2	14.08 $\pm$ 7.2	23.2 $\pm$ 5.3	26.2 $\pm$ 4.31	31.3 $\pm$ 2.5	39.8 $\pm$ 3.6
5th day	2.3 $\pm$ 0.4	9.7 $\pm$ 0.7.7	18.3 $\pm$ 5.2	15.9 $\pm$ 3.8	25.5 $\pm$ 1.8	25.9 $\pm$ 0.7	30.5 $\pm$ 1.3	41.1 $\pm$ 3.7

* Significant decrease, when compared with basal (0 day) measurement ($P < 0.05$)

rats died after deterioration of the health in the period between the measurements made on days one and five. The seven rats that survived were evaluated. In this group, statistically significant differences were observed between the measurements on day zero and day one at frequencies of 8,003 and 10,078 Hz ($P < 0.05$), at 2,003 and 5,039 Hz between day zero and day five ($P < 0.05$), and at 3,175 and 5,039 Hz between day one and day five ($P < 0.05$).

There was no statistically significant difference between the measurements on day zero, day one, and day five in the statistical analysis of Group C and Group D ($P > 0.05$).

Discussion

Cisplatin administration may give rise to hearing problems due to functional and morphological changes in the cochlea. In experimental animal studies, cisplatin reduced the endocochlear potential [29]. Morphologically, cisplatin targets important tissues in the cochlea, including the spiral ganglion, the organ of Corti, and the stria vascularis. One study showed that, in addition to the spiral ganglion and the organ of Corti, cisplatin affected outer hair cells in guinea pigs [30].

Although the mechanism of cisplatin ototoxicity is not well understood, a previous study suggested that the pathogenesis of ototoxicity may be oxidative stress [1]. Following the accumulation of cisplatin in the cochlear tissues as a result of binding DNA and proteins, it inhibits their functions and leads to the production of excessive reactive oxygen radicals, such as superoxide anions. After ROS are produced, nuclear transcription factor-kappa B (NF- κ B), which regulates the expression of proinflammatory cytokines, such as interleukin-1 beta, interleukin-6, and tumor necrosis factor- α (TNF- α), is activated. TNF- α activates NF- κ B, resulting in positive feedback and increasing the inflammatory response in the cochlea. These events can cause apoptosis and a decrease in the number of cells in the cochlea necessary for proper functioning of the inner ear [31, 32].

Many antioxidant agents have been used to prevent cisplatin-induced ototoxicity in experimental animal or clinical trials [4–20, 33–37]. Kizilay et al. [5] performed an experimental study on the protective effect of caffeic acid phenethyl ester (CAPE) on cisplatin ototoxicity and reported that CAPE may have a protective effect. Weissbluth et al. studied the protective effect of systemic dexamethasone administration against cisplatin-induced ototoxicity in a guinea pig model. They observed that dexamethasone slightly decreased TNF- α expression, and they proposed that dexamethasone might be useful in future applications as a complementary treatment for cisplatin-induced ototoxicity [33]. Hyppolitto et al. [34] studied the potential role of sodium salicylate in preventing

cisplatin ototoxicity and providing otoprotection. They demonstrated that salicylates could be used for partial protection of the outer hair cells because of their antioxidant properties. In a study of patients treated with cisplatin, Riga et al. [35] reported that transtympanic injections of *N*-acetylcysteine for the prevention of cisplatin-induced ototoxicity was feasible and showed promising efficacy. Duval et al. [36] revealed that amifostine decreased ototoxicity in patients who received an amifostine infusion prior to cisplatin chemotherapy. However, the results of their meta-analysis of the efficacy of amifostine in preventing cisplatin ototoxicity did not reach statistical significance.

In recent years, naturally occurring antioxidant compounds that are consumed in the diet have gained attention in ototoxicity. Although chrysin is a natural flavonoid that has been the focus of experimental animal studies in recent years due to its strong antioxidant and anti-inflammatory properties, its protective effect against cisplatin ototoxicity has not been previously evaluated. Chrysin exhibits anti-inflammatory properties by inhibiting NF- κ B activation [37]. It was also reported to increase the enzymatic and nonenzymatic antioxidant status, thereby conferring protection against ototoxicity. Previous experimental studies demonstrated that chrysin shows protective efficacy against cisplatin-induced toxicity of various tissues, such as kidney, colon, and jejunum, by attenuating oxidative stress and apoptotic tissue damage [38–40]. Sultana et al. [38] examined the nephroprotective efficacy of chrysin against cisplatin toxicity, administering cisplatin and various concentrations of chrysin to Wistar rats for 14 days. They reported that pretreatment with chrysin attenuated cisplatin-induced renal oxidative damage by diminishing DNA damage and increasing the enzymatic (catalase, glutathione reductase, glutathione-S-transferase, and glutathione peroxidase) and nonenzymatic (reduced glutathione) antioxidant status [38].

The aim of this study was to investigate the protective effect of the antioxidant agent chrysin on the well-known ototoxic effect of cisplatin. No significant differences were noted between the measurements either in the chrysin or in the control groups. The cisplatin group showed a significant worsening of hearing in the measurements at all frequencies on the fifth day compared to baseline. However, in the statistical analysis, a statistically significant difference was observed between the measurements on the first and fifth days only at 5039, 6351, 8003, and 10078 Hz frequencies. In the cisplatin plus chrysin group, there were statistically significant differences between baseline and the measurements taken on the fifth day at frequencies of 2,003 and 5,039 Hz, between the first and fifth days at 3,175 and 5,039 Hz, and between baseline and the first day at 8,003 and 100,078 Hz. In light of these findings, we conclude that cisplatin-induced ototoxicity may be prevented by chrysin administration in rats. However, further

studies that include electrophysiological and histopathological examinations are needed to elucidate the preventive effect of chrysin on cisplatin-induced ototoxicity.

Conflict of interest None.

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