

ORIGINAL RESEARCH

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The protective effect of coumaric acid against cisplatin-induced ototoxicity in rats**Fazile Nur Ekinici Akdemir¹, Mustafa Sitki Gozeler², Serkan Yildirim³, Ahmet Kiziltunc⁴, Gizem Eser³, Seda Askin⁴**¹ Agri İbrahim Cecen University, High School of Health, Department of Nutrition and Dietetics, Agri, Turkey² Ataturk University, Faculty of Medicine, Department of ENT, Erzurum, Turkey³ Ataturk University, Faculty of Veterinary, Department of Pathology, Erzurum, Turkey⁴ Ataturk University, Faculty of Medicine, Department of Biochemistry, Erzurum, Turkey

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Abstract

Cis-diammineedichloroplatinum (cisplatin) is a chemotherapeutic agent used for the treatment of several types of cancer. However, severe side-effects such as ototoxicity, nephrotoxicity and neurotoxicity restrict its use. p-Coumaric acid (PCA) is a phenol class compound obtained from various plants in nature such as grape, carrot and tomato and from beverages such as tea and beer. It was shown to have antioxidant and free radical scavenging, anti-inflammatory and neuroprotective effects. The purpose of this study was to perform a biochemical and histopathological evaluation of the protective efficacy of PCA in ototoxicity induced with cisplatin in rats. To the best of our knowledge, no previous research has investigated the protective effect of PCA against cisplatin-induced ototoxicity. 28 Wistar rats were used. The animals were randomly divided into four groups of seven members each. No drug was administered to the rats in the control group, while the cisplatin group received a single intraperitoneal dose of 10 mg/kg cisplatin. PCA was administered to the PCA group rats by the intraperitoneal route for three days at a dose of 100 mg/kg. In the cisplatin+PCA group, intraperitoneal PCA at 100 mg/kg was administered one hour after injection of 10 mg/kg intraperitoneal cisplatin for three consecutive days. Rats were sacrificed 24 h after the final drug administration. The cochlear tissues were removed and MDA levels, GPx and SOD activities were measured to evaluate the oxidative stress status and histopathological evaluation was performed to reveal cochlear damage. The results showed that PCA protects the cochlea from ototoxic effect of cisplatin. PCA is a reliable agent that provides significant biochemical and histopathological protection against cisplatin-induced ototoxicity in rats.

Keywords: Cisplatin, ototoxicity, coumaric acid**Introduction**

Cis-diammineedichloroplatinum (cisplatin) is a chemotherapeutic agent used for the treatment of several types of cancer. It exhibits its effect by inhibiting DNA synthesis [1]. However, severe side-effects such as ototoxicity, nephrotoxicity and neurotoxicity restrict its use. While the nephrotoxic effect of cisplatin can be prevented with increased hydration and forced diuresis, there is no preventative treatment for its ototoxic effect. Studies have reported a prevalence of ototoxicity of 36% in patients using cisplatin [2]. Besides multiple studies performed on this issue, the mechanism involved in cisplatin-induced ototoxicity is still unclear. However, studies have suggested that an increase occurs in the production of free oxygen radicals (ROS) in the organ of corti, stria vascularis and spiral ligament, and that this causes cochlear damage [3]. Cisplatin also causes overproduction of ROS by inhibiting the antioxidant enzyme systems such as glutathione-S-transferase, glutathione peroxidase and superoxide dismutase [4]. Cisplatin

damages the cochlea by cellular oxidative stress, mitochondrial lysis and peroxidation of lipids [5]. By this way, cisplatin causes oxidative stress as well as blocks the scavenge of the overproduced free radicals by inhibiting the antioxidant enzymes. As a result; bilateral, progressive and irreversible hearing loss, particularly involving high frequencies, occurs in association with cisplatin ototoxicity. This hearing loss worsens depending on the frequency of application and dosage of cisplatin and involves all frequencies [6]. The use of antioxidants against cisplatin-induced ototoxicity draws much attention in recent years because it was showed that one of the main mechanisms is the overproduction of ROS in the cochlear tissues [7].

p-Coumaric acid (PCA) is a phenol class compound obtained from various plants in nature such as grape, carrot and tomato and from beverages such as tea and beer. Studies have shown the antioxidant and free radical scavenging, anti-inflammatory and neuroprotective effects of PCA [8]. Interest in the use of PCA in several diseases has increased due to these properties.

To the best of our knowledge, no previous research has investigated the protective effect of PCA against cisplatin-induced ototoxicity. The purpose of this study was to perform a biochemical and

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histopathological evaluation of the protective efficacy of PCA in ototoxicity induced with cisplatin in rats.

Material and Method

Animals

28 Wistar rats weighing 200 ± 10 g were used. All rats were housed in special cages at a fixed temperature ($22 \pm 10^\circ\text{C}$) and humidity ($55\% \pm 5\%$) with equal day/night cycles and with free access to food and water. Care and Use of Laboratory Animals Guide rules were observed during all procedures.

Experimental Procedure and Induction of Cisplatin: PCA was obtained from the Sigma-Aldrich Chemical Co. and CIS from Kocak Farma Co. (Koçak, Istanbul, Turkey). The animals were randomly divided into four groups of seven members each. An experimental cisplatin-induced ototoxicity model was established. The cisplatin group received a single intraperitoneal dose of 10 mg/kg cisplatin. The rats were sacrificed 72 hours after the administration of cisplatin. PCA was dissolved in 20% ethanol solution and then administered to the PCA group rats by the intraperitoneal route for three days at a dose of 100 mg/kg. In the cisplatin+PCA group, intraperitoneal PCA at 100 mg/kg was administered one hour after injection of 10 mg/kg intraperitoneal cisplatin for three consecutive days. Same volumes of saline were injected to the rats in the control group. Rats were sacrificed 24 h after the final drug administration using 400 mg/kg intraperitoneal sodium thiopental (PENTAL 1G 1 Flakon, İ.E. Ulagay, Türkiye). Cochlear tissues were removed and stored in deep freezers at -800°C for biochemical analyses. Other parts of cochlear tissues were also fixed in 10% formaldehyde for histopathological examination.

Biochemical Analysis

Rats were sacrificed with high-dose anesthesia and the cochlear tissues of the rats removed and rapidly washed with physiological saline and then frozen. The cochlear tissues obtained from each rat were ground in liquid nitrogen with the assistance of a commercially available Tissue Lyser II grinding jar kit (Qiagen, Hilden, Germany). All tissue specimens were homogenized with 9 ml per gram 1.15% KCL buffer solution. Supernatant was obtained by centrifuging this homogenate for 30 min at 10000 rpm. MDA level, GPx and SOD activity were calculated from each supernatant sample using an ELISA reader [9-11], after which mean absorbance values were measured. A standard curve was calculated, and the data elicited from the absorbance of standards were used to calculate proportions of GPx, MDA and SOD. Tissue SOD and GPx activity and MDA levels were expressed as U/ml, iU/L, and nmol/ml, respectively. Levels of proteins were determined using the Lowry method. Bovine serum albumin was used as the standard commercial protein (total protein kit-TP0300-1 KT; Sigma Chemical Co., Munich, Germany).

Histopathological Analysis: Both temporal bones were removed from all rats after sacrifice, and the cochleas were isolated. The cochlear tissues were fixed in 10% formalin solution for 48 h for histopathological investigation. After softening for 96-120 h in Osteosoft (Merc, HC313331, Germany) decalcification solution, specimens were washed for 24 h in running tap water. After being passed through 80% alcohol (12 h x 2 times), 90% alcohol (12 h

x 2 times), 96% alcohol (12 h x 2 times), and 100% alcohol (12 h x 2 times), chloroform (5 h x 3 times), and liquid paraffin (12 h), the specimens were embedded in paraffin blocks. Sections 4 μm in thickness were taken from each block, and preparates were readied on slides. The preparates produced for histopathological examination were stained with hematoxylin and eosin and studied under light microscopy. Sections examined under light microscope were assessed on the basis of lesions as none (-), mild (+), moderate (++) or severe (+++), and photographs were taken.

Statistical Analysis: Data were analyzed on SPSS 17.0 software. Distribution at data comparison was assessed using the Shapiro-Wilks test. One-Way ANOVA was used since data were normally distributed. The Post-Hoc Tukey test was applied to determine the source of variance. The non-parametric Kruskal-Wallis test was used to analyze differences between groups in terms of semiquantitative data at histopathological examination, and the Mann Whitney U test for two-group analyses. The results were expressed as mean $\pm$ standard deviation and $p < 0.05$ was accepted as statistically significant.

Results

Biochemical Results

Biochemical changes occurring in cochlear tissues are summarized in Figure 1.

MDA levels, a parameter of lipid peroxidation, were significantly higher in the cochleas of rats receiving cisplatin than in the control group. MDA levels in rats receiving cisplatin together with PCA were significantly lower than in rats receiving cisplatin alone. Administration of PCA exhibited a protective effect against cisplatin-induced MDA elevation.

Activities of SOD and GPx, both components of the antioxidant enzyme system, were significantly lower in the cochleas of rats receiving cisplatin compared to the control group. Both SOD and GPx activities were significantly higher in rats receiving PCA together with cisplatin compared to rats receiving cisplatin alone. Administration of PCA also had a protective effect against a decrease in antioxidant enzymes caused by cisplatin.

Histopathological Findings

The histopathological differences were summarized at Table 1. In control group, the stria vascularis, organ of Corti and spinal ganglion exhibited a normal histological structure (Figure 2). On the other hand, vessels in the stria vascularis were dilated and congested, and desquamation and erosion were observed in the cisplatin group (Figure 3A). Also there were a severe decrease in outer hair cell numbers in the organ of Corti (Figure 3B) and degeneration and necrosis was determined in spinal ganglion cells (Figure 3C).

The rat in pCA group also showed a normal histological appearance in the stria vascularis, organ of Corti and spinal ganglion (Figure 4). There were mild dilation and congestion in the vessels of the stria vascularis in rats received Cisplatin+PCA (Figure 5-A). Also, a moderate decrease was observed in outer hair cell numbers in the organ of Corti (Figure 5B) and mild degeneration was determined in spinal ganglion cells (Figure 5C).

Table 1. Cochlear histopathological findings

	Number of outer hair cells in the organ of Corti	Desquamation and erosion in the stria vascularis	Dilation and congestion in the stria vascularis	Degeneration and eosinophilic necrosis in the spiral ganglion
Control	+++	-	-	-
Cisplatin	+	+++	+++	+++
pCA	+++	-	-	-
Cisplatin+pCA	++	+	+	-

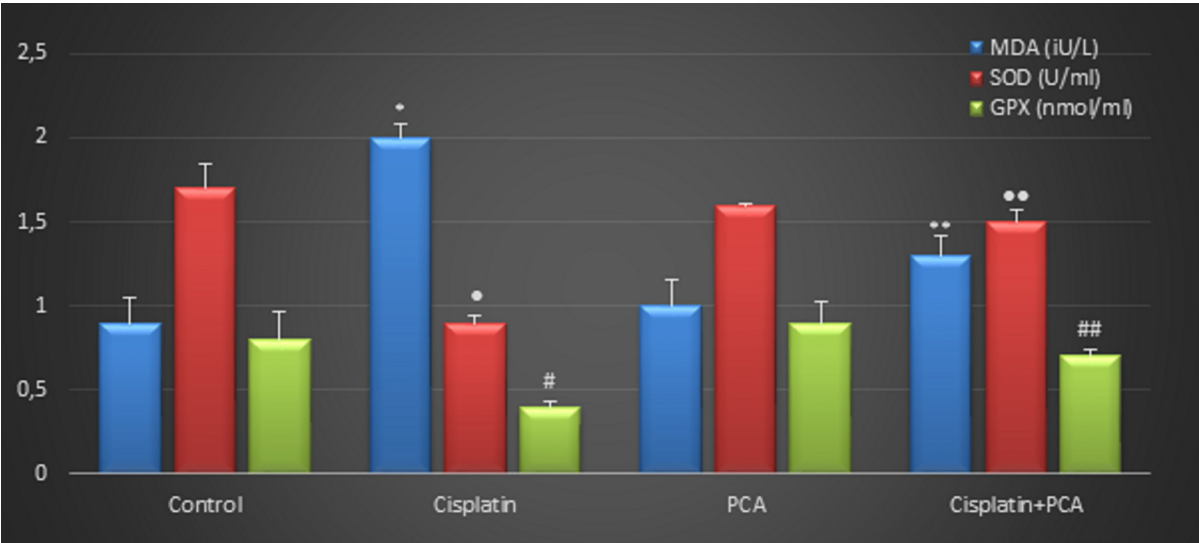


Figure 1. The results of MDA levels, GPx and SOD activities of cochlear tissues
*: Statistically significant in the level of $p<0.05$ between cisplatin and control groups. **: Statistically significant in the level of $p<0.05$ between Cisplatin+PCA and cisplatin groups. •: Statistically significant in the level of $p<0.05$ between Cisplatin and Control groups. ••: Statistically significant in the level of $p<0.05$ between Cisplatin+PCA and cisplatin groups. #: Statistically significant in the level of $p<0.05$ between Cisplatin and control groups. ##: Statistically significant in the level of $p<0.05$ between Cisplatin+PCA and cisplatin groups.

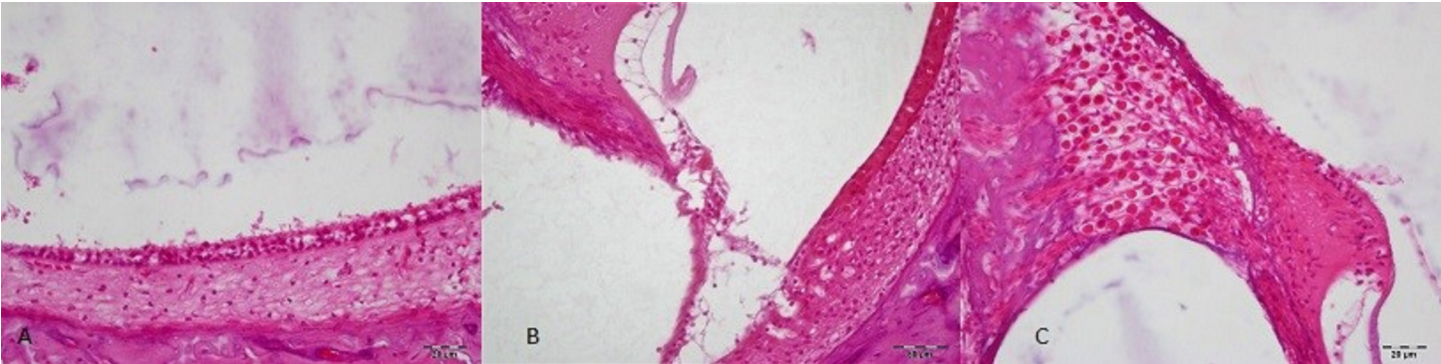


Figure 2. Normal histopathological structure of the cochlea. A: Stria vascularis, B: Organ of Corti, C: Spiral ganglion

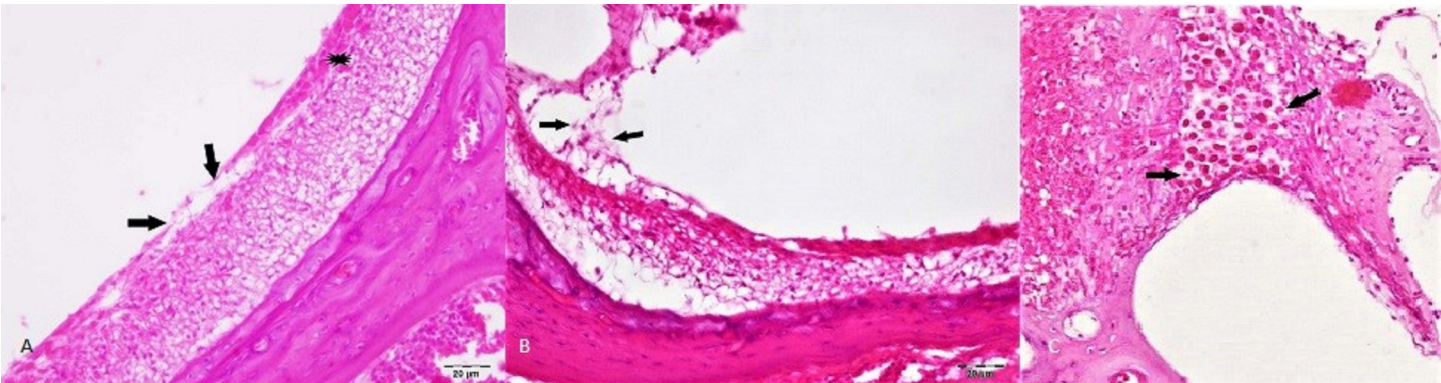


Figure 3. Cochlear structure in rats receiving cisplatin. A: Desquamation and erosion (arrows) and dilation and congestion of vessels (asterisk) in the stria vascularis. B: A severe decrease in the number of outer hair cells in the organ of Corti (arrows). C: Degeneration and necrosis in cells of the spiral ganglion (arrows).

Discussion

In this study, we determined the protective effect of pCA against the harmful effect of cisplatin on cochlea. It was shown that the MDA levels were lowered as well as the SOD and GPx levels were increased in the cochlear tissues of rats received pCA together with cisplatin. Thus we found that pCA prevents the oxidative stress caused by cisplatin in the cochlea of the rats. Also, the histopathological evaluation of the cochlea revealed that the damages on the organ of corti, stria vascularis and spiral ganglion caused by cisplatin were all protected by treatment of pCA. As a result, it was suggested that pCA has a protective effect against cisplatin induced ototoxicity in term of biochemical and histopathological aspect.

Cisplatin-induced ototoxicity is characterized by progressive irreversible and generally bilateral hearing loss and is related to the dose administered and the frequency thereof. The pathogenesis is still unclear, but the main underlying mechanism is damage occurring in outer hair cells of the cochlea. This injury is thought to be caused by increased production of free oxygen radicals in the organ of Corti, stria vascularis and spiral ligament and a decrease in the antioxidant defense system [12]. The first injury in the cochlea in cisplatin-induced ototoxicity appears in the stereocilia of the outer hair cells, followed by loss of outer hair cells from the basal portion to the apex. The damage also leads to collapse in Reissner's membrane and to atrophy in supporting cells in the stria vascularis and organ of Corti [13]. Studies involving various antioxidant substances have been performed in terms of preventing this ototoxicity. Experimental studies of cisplatin-induced ototoxicity have investigated the protective efficacy of various antioxidants such as resveratrol, vitamin E, N-acetylcysteine, sodium salicylate, melatonin and pomegranate [14-19]. However, despite all these studies, there are no FDA-approved agents for the prevention of cisplatin-induced ototoxicity. This study investigated the effect of PCA use in preventing ototoxicity induced with cisplatin in rats. Our findings show that PCA prevents cochlear injury occurring in ototoxicity and increases antioxidant levels in the cochlea.

PCA is a phenolic acid and member of the hydroxycinnamic acid family. It occurs widely in mushroom, and various fruits (including apples, pears, grapes, oranges, and tomatoes), vegetables (including potatoes and onions) and cereal crops (such as maize, oats and wheat). PCA exhibits antioxidant, anti-inflammatory, anti-ulcer, antiplatelet, anti-carcinogenic and anti-mutagenic activities [20]. Kim et al. investigated the efficacy of PCA against scopolamine-induced learning and memory disturbance. They reported that at a dosage of 30 mg/kg PCA improved impairment of electrophysiological and cognitive functions in rats and exhibited a regulatory effect on the central cholinergic synapses. At the same time, they suggested that PCA can also improve cognitive problems deriving from cholinergic nervous system anomalies. They thus identified PCA as a neuroprotective agent [21].

Moneim et al. investigated the effect of PCA against neurodegeneration in rats. They showed that PCA significantly improved glucose tolerance, reduced oxidative stress in the brain, increased antioxidant levels and inhibited apoptosis against diabetes-related neurodegeneration. They concluded that PCA inhibited hippocampal neurodegeneration with its powerful antioxidant, anti-inflammatory and anti-apoptotic properties [22].

Sharma et al. investigated the effect of PCA on chronic preneoplastic lesions and concluded that it significantly inhibited these lesions. PCA protects the intestinal system against genotoxic attacks by inhibiting these lesions with its powerful antioxidant property, free radical scavenging activity and detoxification mechanism. They also compared the efficacy of PCA at three different doses and reported that 100 mg/kg was the most effective of these [20]. In our study we also used PCA at a dosage of 100 mg/kg. Zang et al. investigated the effect of PCA on LDL cholesterol oxidation and reported that PCA used orally significantly inhibited LDL oxidation. They also showed that PCA reduced LDL cholesterol levels without affecting HDL cholesterol [23]. Akdemir et al. investigated the protective effect of PCA on cisplatin-induced acute liver and kidney injury. They used cisplatin to induce acute liver and kidney in adult Wistar rats and reported that PCA prevented such damage [24].

The most important limitation of our study is that clinical tests such as Distortion Product Otoacoustic Emissions and Auditory Brainstem Response capable of demonstrating the functional damage in the cochlea in rats were not employed.

Conclusion

PCA is a reliable agent that provides significant protection against cisplatin-induced ototoxicity in rats. This paper is the first to demonstrate such efficacy. Further and broad-based studies are now needed to prove the applicability of this protective effect in humans.

Competing interests

The authors declare that they have no competing interest

Financial Disclosure

There are no financial supports.

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