Original Article

GSTP1 Levels in Cisplatin-induced Rat Cochlea after Alpha Lipoic Acid and Oxytocin Treatment

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Abstract

Introduction: Cisplatin is a well-known chemotherapeutic agent used in many cancer treatments. Several antioxidant agents are used for diminishing the toxic side effects of the cisplatin therapy. Alpha-lipoic acid (α-LA) and oxytocin (OT) are antioxidant agents that can be used in toxicity. Our aim is to investigate the effect of these antioxidants in cisplatin-induced ototoxicity in tissue level. **Materials and Methods:** Forty Wistar albino rats divided into five groups as control, cisplatin, cisplatin + intraperitoneal (IP) OT, cisplatin + intratympanic (IT) OT, and cisplatin + IT α-LA. The drug administration is applied for 4 days, and at the end of the procedure, the cochleas are harvested. After tissue preparation, *GSTP1* levels are investigated and the intensity of the reaction is scored as negative (−), weak (1+), moderate (2+), or strong (3+). **Results:** Group 4 has a moderate staining which can be interpreted as high immunoreaction. When we compare with Group 1, this staining difference is statistically significant ($P < 0.02$). When we observe the Group 3, we cannot detect any difference with Group 1 in immunoreactivity. **Conclusion:** α-LA and OT are antioxidants effective against cisplatin ototoxicity. The expression of *GSTP1* isozyme is increased in antioxidant-treated groups. Increased levels of these isozymes proved the increased healing response in tissue levels. Antioxidant agents can be used for adverse effects during cisplatin treatment. IT route is effective as IP systemic route.

Keywords: Alpha-lipoic acid, cisplatin, *GSTP1*, ototoxicity, oxytocin

Introduction

Cisplatin, which is widely used well-known chemotherapeutic agent, has potential various side effects. The mechanism of this undesirable side effect is searched detailly, and several theories are defined on the last decades. These studies mostly focused on the cochlea structural epithelium and specialized cells such as spiral ganglion, organ of corti, and outer and inner ear cells. The cochlea comprises metabolically active tissues that can synthesize reactive oxygen species (ROS). By this way, these valuable cells mostly destroyed by the product of ROS such as nitric oxide (NO), superoxide radical (O_2−), peroxynitrite (ONOO−), S-nitrosothiols (RSNOs), and hydrogen peroxide (H_2O_2). ROS products which are toxic products leading to calcium influx promote the cycle of apoptosis.[1]

Two types of antioxidant enzymes have role in the cochlea. One of these enzymes involved in glutathione (GSH) metabolism such as glutathione S-transferase (GST), GSH peroxidase, and glutathione reductase. The second enzymes involved in the breakdown of superoxide anions and H_2O_2 such as catalase and Cu/Zn superoxide dismutase (SOD1).[2,3]

GSTs represent a family of ubiquitous cytosolic enzymes that play a physiological role in the detoxification of alkylating and platinating agents. These enzymes break down electrophilic substrates by catalyzing the conjugation of toxic substrates with the sulfhydryl group of GSH.[4] This neutralizes the electrophilic sites and renders the products more water soluble. Touliatos *et al*. showed that GST was expressed throughout the rat cochlea, with cisplatin treatment causing its decreased expression.[5] Oldenburg *et al*. found that the presence of both alleles of 105Val-*GSTP1* offered protection against cisplatin-induced hearing impairment, and two genotype patterns with good

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and poor protection against cisplatin-induced ototoxicity were identified.[6]

Alpha-lipoic acid (α-LA) is kind of a powerful antioxidant and anti-inflammatory agent with a dithiolane ring. Moreover, α-LA has an active metabolite named dihydrolipoic acid. Besides these roles in cellular level, α-LA is also a cofactor for mitochondrial enzymes, metal chelator, and free radical scavenger.[4] α-LA regenerates GSH, coenzyme Q10, ascorbate, and Vitamin E directly and indirectly.[7] α-LA is able to fight both intracellular and extracellular formed free radicals in any part of the cell. Furthermore, α-LA improves cellular metabolism and the healing process.[7]

Oxytocin (OT), which is a well-known hormone, is synthesized increasingly in blood during pregnancy. This hormone was investigated previously, and the protective effect of OT on cisplatin nephrotoxicity by its antioxidant and anti-inflammatory effect was showed.[8,9] OT reduces consumption of GSH and SOD, inhibits NADPH oxidase and myeloperoxidase, elevates NO levels, and prevents inflammation and apoptosis.[10-12]

Our aim is to identify the GST isozyme expression in rat cochlea which is treated with cisplatin, cisplatin with LA, and cisplatin with OT.

Materials and Methods

This study was carried out at the Marmara University Experimental Medical Research Institute's Animal Laboratory and was approved by Marmara University Local Ethics Committee for Animal Experiments. The study included 40 healthy adult female and male albino rats with a normal ear canal and tympanic membrane. The animals weighed 190–300 g (mean weight: 240 g), and the rats were housed in an environment with a light cycle (12 h light, 12 h dark) at 25°C temperature. The animals were fed standard diet and water. Following induction of anesthesia with 90 mg/kg of intramuscular ketamine hydrochloride (Ketalar; Pfizer, New York, USA) and 10 mg/kg of xylazine (Rompun; Bayer, Leverkusen, Germany), the external ear canal and eardrum were examined by an operating microscope.[4,13]

Study design

The animals were divided into five groups, including a control group (Group 1), cisplatin-saline group (Group 2), and cisplatin-İT α-LA group (Group 3), cisplatin-intratympanic (IT) oxytocin group (Group 4), and cisplatin-intraperitoneal (IP) oxytocin group (Group 5). Then, 0.9% saline was administered to the Group 1 intratympanically and intraperitoneally for 4 days (8 animals, 16 ears). Group 2 received a 20-mg/kg cumulative dose (10 mg dose for 2 days) of IP cisplatin (Cisplatin; Koçak Pharma, İstanbul, Turkey) and IT saline solution for 4 days (8 animals, 16 ears). Group 3 received IP cisplatin with the same protocol and 25 mg/mL of IT α-LA (Thioctacid; Meda Pharma, Bad Homburg, Germany) solution 30 min before cisplatin administration for 4 days (8 animals, 16 ears). Group 4 received the same dose of cisplatin and IT OT (Synpitan fort 5 IU/5 ml amp, Deva Ilaç, Turkey) for 4 days (8 animals, 16 ears), and Group 5 received the same dose of cisplatin and IP OT with dose of 1 mg/kg, as an otoprotective agent, for 4 days (8 animals, 16 ears). Transtympanic injections were done with a 28-gauge needle through the anterosuperior quadrant of the tympanic membrane. The injection was continued for about 15 s until the liquid was observed filling the middle ear and coming back to the outer ear (approximately 0.2 mL). Transtympanic injections were done 30 min before cisplatin injections to presumably allow a reasonably high concentration of drugs in the inner ear. At the end of the 5th day, the same anesthesia protocol was applied and animals were decapitated. All the cochleae of the groups were harvested, and histopathological examination was conducted.

Tissue preparation

Tissues were fixed in 10% buffered formalin and embedded in paraffin blocks. Sections that were 4 μm thick were cut, and one section was stained with hematoxylin-eosin to observe the tissue morphology. For immunohistochemistry, endogenous peroxidase activity was blocked by incubating the sections in 3% H_2O_2 (v/v) in methanol for 10 min at room temperature (RT). The sections were subsequently washed in distilled water for 5 min, and antigen retrieval was performed for 3 min using 0.01M citrate buffer (pH 6.0) in a domestic pressure cooker. The sections were transferred in 0.05M Tris-HCl (pH 7.6) containing 0.15M sodium chloride (TBS). After washing in water, the sections were incubated at RT for 10 min with super block (SHP125) (ScyTek Laboratories, USA) to block nonspecific background staining. The sections were then covered with the primary antibodies diluted 1:100 for anti-*GSTP1* (Santa Cruz Biotechnology, cat no sc-28494) were from Abcam Inc., USA, in antibody diluent solution at 4°C overnight. After washing in TBS for 15 min, the sections were incubated at RT for biotinylated link antibody (SHP125) (ScyTek Laboratories, USA). Then, treatment was followed with streptavidin/HRP complex (SHP125) (ScyTek Laboratories, USA). Diaminobenzidine was used to visualize peroxidase activity in the tissues. Nuclei were lightly counterstained with hematoxylin, and then, the sections were dehydrated and mounted. Both positive and negative controls were included in each run. Positive control consisted of section of liver tissues for *GSTP1*. TBS was used in place of the primary antibody for negative controls.

Light microscopy of immunohistochemically stained sections was performed by a pathologist and a biologist, who were unaware of clinical information. Distribution, localization, and characteristics of immunostaining were recorded. Brown color in cytoplasm and/or nucleus of epithelial cells of the cochlea was evaluated as positive staining. Scoring was also performed by observers unaware of the information. Scoring differences between observers were resolved by consensus. For each antibody, the intensity of the reaction – negative (−), weak (1+), moderate (2+), or strong (3+) – was determined to describe the immunoreactions.

Results

Figure 1 has shown the morphology of the cochlea. The *GSTP1* immunohistochemical staining for all groups has shown in Figure 2a-e. Group 4 has a moderate staining which can be interpreted as high immunoreaction. When we compare with Group 1, this staining difference is statistically significant ($P < 0.02$) [Table 1]. Group 2 also has higher immunoreactivity than Group 1, but this difference is not statistically significant. When we observe Group 3, we cannot detect any difference with Group 1 in immunoreactivity. Group 5 has weak immunoreactivity which is also higher than Group 2.

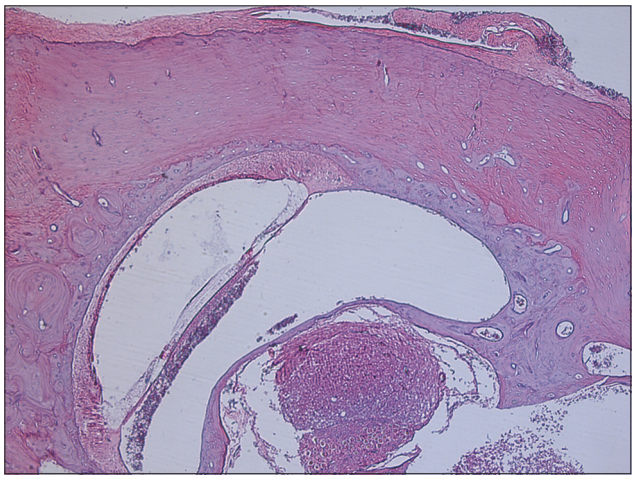

Figure 1: Epithelial cells in the cochlea (H and E, ×5)

Discussion

Oncologic diseases are treated with chemotherapeutic agents such as cisplatin although they have serious devastating side effects. Some of these side effects are nephrotoxicity, neurotoxicity, and ototoxicity which may also limit the cancer treatment dosage. The characteristic feature of the ototoxicity is sensorineural hearing loss bilaterally in higher frequencies at the beginning and followed by lower frequencies whether the treatment continued.[14] Cisplatin causes ototoxicity in the cochlea in three different area of the cochlea, hair cells in the basal turn of the organ of corti, spiral ganglion cells, and lateral wall tissues such as spiral ligament and stria vascularis. Spiral ligament, outer hair, and stria vascularis cells are affected with the platinated DNA immunoreactivity by cisplatin, resulted with the apoptosis of these valuable cochlear cells.[14] In addition, ROS, which are formed during the cisplatin treatment, lead to several devastating effects on these cells. Antioxidant agents are used against cisplatin ototoxicity. These agents are used depending on the principle of chelating effect to ROS materials. Antioxidants also increase the GSH enzymes which increase the neutralization of the ROS product. Ability of antioxidant system is important in healing

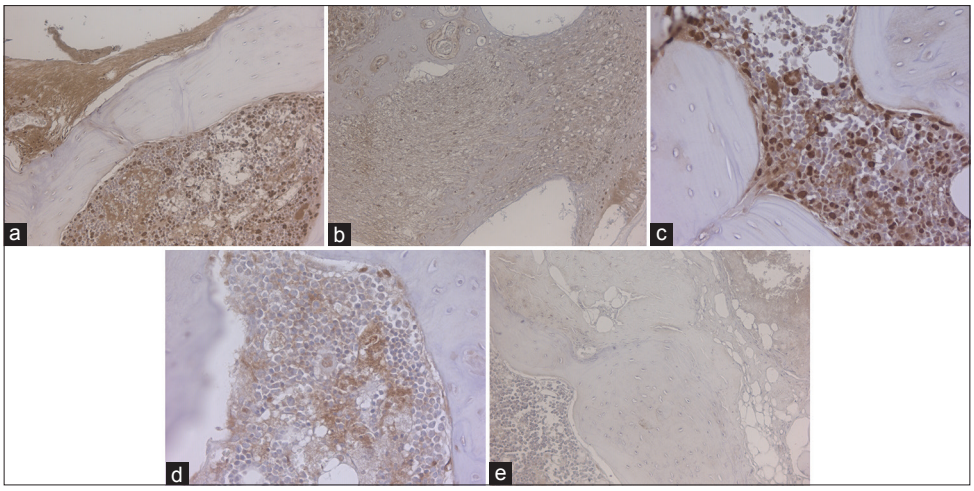

Figure 2: (a) *GSTP1* moderate staining in epithelial cells of the cochlea (Group 4, ×20). (b) *GSTP1* weak staining in epithelial cells of the cochlea (Group 5, ×20). (c) *GSTP1* moderate staining in epithelial cells of the cochlea (Group 2, ×40). (d) *GSTP1* weak staining in epithelial cells of the cochlea (Group 1, ×40). (e) *GSTP1* weak staining in epithelial cells of the cochlea (Group 3, ×10)

Table 1: Comparison of expression of GSTP1 gene levels between control and other metabolites

	Mean	SD	SEM	95% CI of the difference		*t*	*P*
				Lower	Upper		
Control-oxytocin	−1.50000	0.63246	0.25820	−2.16372	−0.83628	−5.809	0.002*
Control-lipoic acid	0.10000	0.75366	0.30768	−0.69092	0.89092	0.325	0.758
Control-oxytocin IP	−0.30000	0.40000	0.16330	−0.71977	0.11977	−1.837	0.126
Control-cisplatin	−0.60000	1.04115	0.42505	−1.69262	0.49262	−1.412	0.217

Paired *t*-test results are shown. SD: Standard deviation, SEM: Standard error of mean, CI: Confidence interval, IP: Intraperitoneal

property in ototoxicity. On the light of this mechanism, some genetic factors are investigated to clarify the susceptibility to cisplatin ototoxicity. It is shown that the presence of both alleles of 105Val-*GSTP1* appeared to offer protection against hearing loss from cisplatin.[13]

The expression of GST enzyme levels is investigated for cisplatin treatment. This study concluded that animals treated with cisplatin treatment showed lower GSTA staining in all regions of the cochlea when compared with normal controls. They cannot explain whether this is related with consumption of the GST enzymes or blockage to expression of the GST enzymes. Stria vascularis which is the richest area of blood supply has the lowest level of GST enzymes and also first affected area in cisplatin ototoxicity.[5] Previous studies revealed that increased level of GST in tumor cells protects the cisplatin effect.[15] In our study, we have seen increased expression of *GSTP1* on OT which applied with IT route. IP systemic route of OT is lower than local route. We have seen that systemic elimination diminished the tissue response in enzyme levels. When we measure the *GSTP1* levels in LA group, we can detect the low immunoreaction levels nearly same as control group. We can conclude that *GSTP1* isozyme levels are not affected with LA. Platinum based drugs are the substrate of the GSTP isozyme gene family, so this may be another reason of the decreased response.

Lautermann *et al*. investigated the cisplatin treatment on cochlear damage in rats. They have shown the decreased level of GSH levels when compared with normal controls. This decrease can be detected even in lower dose (1 mg/kg) of cisplatin treatment.[16] In our study, we applied 20 mg/kg cumulative dose of cisplatin to accomplish cochlear toxicity. Moreover, we proved this toxicity by measuring the otoacoustic emission before and after cisplatin administration.[17,18] Application of IT route is effective way of drug administration and also diminishes both systemic elimination and side effects. We advise these drugs in ototoxicity due to cisplatin usage. Testicular cancer survivors who are treated with cisplatin treatment investigated about the GST gene expressions. The study concluded that genotype 105Val/105Val-*GSTP1* is protective against cisplatin-induced ototoxicity.[6] In our study, we have detected the increased levels of *GSTP1* isozyme levels that support the previous reports.

Conclusion

The cisplatin therapy, which is used in various cancers, can cause toxicities in tissue levels. We can detect this response in biochemistry tissue analysis. Protective antioxidant agents can protect the adverse effects of the cisplatin treatment. Both LA and oxytocin are effective against cisplatin ototoxicity. Increased level of *GSTP1* isozyme can be seen in this antioxidant therapy. We advise LA and oxytocin during cisplatin therapy.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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