The Effect of Beta Glucan on Cisplatin Ototoxicity

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Abstract This study was undertaken to investigate the effect of betaglukan in ameliorating cisplatin ototoxicity. Rats were divided into four groups: cisplatin (C), cisplatin plus beta glucan (CB), beta glucan (B), and control (K). Distortion product otoacoustic emissions were elicited in 0th, 1st, and 5th days. For the group C differences were observed at 8,003 and 9,515 Hz between 0th and 5th days' measurements. In the group CB there were differences at frequencies of 3,996, 4,757, 5,660, and 6,726 Hz between 0th and 5th days' measurements. For the group B there were significant recovery in some frequencies. The observation of significant deterioration in terms of hearing in the group treated with cisplatin plus betaglukan may be suggested that depended on the increase of permeability and tissue conductance into the inner ear which may be caused by betaglukan. Further

long-term follow-up studies by using different doses may clarify this matter.

Keywords Ototoxicity · Cisplatin · Betaglukan · Otoacoustic emissions

Introduction

Cisplatin (cis-diamine-dichloroplatinum), is an effective antineoplastic agent widely used to treat cancer of many organs, including cancers of the head and neck region. However, the side effects and toxicity may be seen, even the standard dose was administered, due to not target tumor cells specifically. The toxicity of cisplatin is dose-dependent and it is known that the usage of high doses increase mortality and morbidity such as ototoxicity or nephrotoxicity [1]. Although nephrotoxicity, may effectively decreased by intravenous hydration and diuresis, ototoxicity still poses a major limitation to effective cisplatin chemotherapy [2, 3]. Although the mechanisms of cisplatin ototoxicity are not fully understood, reactive oxygen species have been implicated in its pathogenesis.

Beta glucan is a heterogeneous polymer of glucose which is present in the cell wall of yeasts, cereals and fungi. It is known that, it is a good anti-oxidant as well as has many important biological activities [4]. Antioxidants are the substances that prevent or limit oxidative damage by inactivating the oxidants in cells. The purpose of this study was to investigate the protective effect of betaglukan, which is known to be a powerful antioxidant agent, against ototoxicity induced by cisplatin by using DPOAE measurements.

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Methods

After our study has been approved by the Experimental Animal Ethics Committee, 40 three-month of age male Wistar albino rats weighting 200–280 g were used for the present study. The rats were housed in a place where they can feed ad libitum for 12 h in light and 12 h in dark, at 21 °C temperature. The external ear canals and tympanic membranes of the animals were examined with otomicroscopy (Fig. 1). The otoacoustic emission measurements were performed in special designed quiet cabin made by using glass and wool in which noise level does not exceed 45 dB sound pressure level (Fig. 2). The rats with normal DPOAE findings were included in the study.

The animals were separated into four groups: cisplatin (C), cisplatin plus betaglukan (CB), betaglukan (B) and control (K). For group C, 16 mg/kg/intraperitoneal (i.p.), single dose cisplatin at 0th day; for group CB, 16 mg/kg/i.p. single dose cisplatin at 0th day and 1 mg/kg/day beta glucan between 0th and 5th days with lavage; for group B, 1 mg/kg/day beta glucan between 0th and 5th days with gavage were given. Group K was followed without administration.

The measurements DPOAE were performed by using GSI Audera DPOAE (Grason Stadler, Madison, USA) device before administering the medicine (0th day) and at 1st and 5th days after administration of the medicine. The placement and calibration of the probe was made by measuring system automatically before the test. For DP grams measurements, primer stimulus levels were equalized at 65 dB ($L_1 = L_2$), two different frequencies (f_1 and

Fig. 2 Special quiet cabin used for DPOAE measurements

f_2) were organized as $f_2/f_1 = 1.22$ that the most powerful responses may have been taken. Measurements were performed at frequencies of 2,003, 2,378, 2,824, 3,363, 3,991, 4,757, 5,660, 6,726, 8,003, 9,515 Hz, and the results were recorded. Measurements were performed under anesthesia using a combination of ketamine hydrochloride (30 mg/kg) and xylazine (6 mg/kg).

SPSS for Windows Version 13 program was used for statistical evaluation. Measurable variables are expressed as mean $\pm$ standard error (SEM). The Mann–Whitney U test was used for comparing groups; the Wilcoxon two-sample paired signed rank test was used for the changing's inside the groups. $P < 0.05$ was considered as statistically significant.

Results

One of the 12 rats in group C did not wake up after anesthesia during the measurements of the 1st day and four rats died after deterioration of the general situation in the period between the measurements of 1st and 5th days. Seven rats were able to complete the study and evaluated. For the group cisplatin administered, although there is a significant worsening of hearing compared to 0th and 5th days' measurement at all frequencies; in statistical analysis, statistically significant difference was observed at 8,003 and 9,515 Hz between 0th and 5th days' measurements ($p < 0.05$) (Fig. 3).

Two of the 12 rats in CB group did not wake up after anesthesia in the first day's measurements, four rats died on different days after the deterioration of general situation. In this group administered cisplatin plus betaglukan, six rats were able to complete the study and evaluated. Especially at frequencies 3,996 Hz and above, compared to the measurements of 0th and 5th day, significant worsening of

Fig. 1 Otomicroscopic examination of the external ear canal and tympanic membrane

Fig. 3 Variations in amplitudes of distortion products otoacoustic emissions with frequency (F_2) for different time points in cisplatin used group. (CB baseline (0th), C1; 1st day, C5th day measurements)

hearing was determined, and there were statistically significant differences at frequencies of 3,996, 4,757, 5,660, and 6,726 Hz between 0th and 5th days' measurements ($p < 0.05$) (Fig. 4).

Only one rat from the group beta glucan administered was excluded from the study due to not woke up after anesthesia and seven animals were included in evaluation. In this group, at 2,378, 2,824, 3,363, and 3,996 Hz, between the measurements of 0th and 1st days, also 8,003 and 9,515 Hz, between the measurements of 0th and 5th days were statistically significantly different in terms of hearing recovery ($p < 0.05$) (Fig. 5).

There was no statistically significant difference between the measurements of 0th, 1st, and 5th days of the statistical analysis of K group ($p > 0.05$).

Discussion

The serious side effects of cisplatin, ototoxicity, nephrotoxicity, and neurotoxicity vary depending on dose, frequency and duration of usage in the majority of patients [5, 6]. Hearing loss is closely depending on cisplatin dose and the frequency of usage. In adults, ototoxic damage is seen more particularly with usage of high doses [7]. Cisplatin related nephrotoxicity can be reduced with intravenous hydration and the provision of diuresis. However, any method can be used to reduce ototoxicity is not known yet [1, 8]. Ototoxicity is bilateral, permanent, sensorineural

type, initially involves only the high frequencies and in time also affects low frequencies [9].

Although the pathogenesis of cisplatin ototoxicity has not been elucidated clearly yet, it is believed to be due to the formation of reactive oxygen species [10]. It was demonstrated that administration of cisplatin was resulted in a decrease in antioxidant enzymes and glutathione and an increase in the malondialdehyde level at cochlea [11]. Decrease in cochlear glutathione, glutathione peroxidase and reductase levels, increase in superoxide dismutase, catalase activity and malondialdehyde levels were observed with the administration of cisplatin, therefore it is accepted that, these changing of antioxidant enzyme activities were responsible for cochlear injury and ototoxicity [10].

Antioxidants are the substances that protect cells against the side effects of drugs, carcinogens and toxic radical reactions both directly and indirectly [12, 13]. Today, many antioxidants are used for therapeutic purposes. Recombinant superoxide dismutase, iron chelators, allopurinol, E-C-A vitamins, beta-carotene, methionine, caffeic acid phenethyl ester, glutathione, probucol, erdostein, aminoguanidine, and angiotensin converting enzyme inhibitors can be given as examples [14–16]. Also, there are some studies in which betaglucon known as an effective antioxidant has a protective effect against nephrotoxicity induced by cisplatin has been reported [17].

In this study performed with beta glucan which antioxidant and immunomodulatory effects are known, for the

Fig. 4 Variations in amplitudes of distortion products otoacoustic emissions with frequency (F_2) for different time points in cisplatin and beta glucan used group. (CBB baseline (0th), CB1; 1st day, CB5; 5th day measurements)

Fig. 5 Variations in amplitudes of distortion products otoacoustic emissions with frequency (F_2) for different time points in beta glucan used group. (BB baseline (0th), B1; 1st day, B5; 5th day measurements)

group used cisplatin there are statistically significant differences in terms of hearing loss at two frequencies between 0th and 5th days' measurements. For the group used cisplatin with beta glucan, there are statistically significant differences in terms of hearing loss at four frequency between 0th and 5th days' measurement.

The immunomodulatory effect of beta glucan is known [4]. In a recent study, it had been showed that, beta glucan increased the mannitol permeability and tissue conductance in pigs with barley-based diet fed, especially in high beta glucan contain group compared with control group [18]. Therefore, on the bases of this study, it may be considered that beta glucan increases the penetration of cisplatin to the inner ear.

In the current study, similarly with our previous study [19], the improvement of hearing determined between 0th and 5th days' measurements at 3,363 and 8,003 Hz; and between 1st and 5th days' measurements at 8,003 for the group only beta glucan administered may be associated with the immunomodulatory effect of beta glucan.

In the lights of this knowledge and the results of our studies, further investigations are needed to expose the effect mechanism of beta glucan, especially dose-dependent.

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