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Analysis of genetic and non genetic risk factors for cisplatin ototoxicity in pediatric patients

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ABSTRACT

Objective: The aim of this study was to analyse the genetic and non genetic risk factors for cisplatin ototoxicity.
Methods: This study was conducted on 72 children who received cisplatin based chemotherapy. Brock and Muenster classifications were used to evaluate ototoxicity seen in these children. 6 single nucleotide polymorphisms (SNP); *ERCC1* rs 11615, *GSTP1* rs1138272, *GSTP1* rs1695, *LRP2* rs 2075252, *TPMT* rs 12201199, *COMT* rs 9332377, were evaluated as genetic factors by real time PCR. Non genetic factors such as cranial irradiation, cumulative doses of cisplatin, age, gender, administration of other ototoxic drugs were analysed as well. By using Chi-square test, risk factors were matched with the ototoxicity classifications. Significant risk factors were reevaluated using logistic regression modelling.
Results: According to univariate analyses, male gender, co-treatment with aminoglycosides and mutant genotype of *GSTP1* rs1695 were significantly related with cisplatin ototoxicity. Logistic regression modelling analyses also showed that male gender, co-treatment with aminoglycosides were found to be significantly related with cisplatin ototoxicity. Mutant genotype of *GSTP1* rs1695 was not found to be significant, but close to the level of statistical significance.
Conclusion: Male gender, co-treatment with aminoglycosides are significant risk factors for cisplatin ototoxicity in pediatric patients. Mutant genotype of *GSTP1* rs1695 seems to be a genetic risk factor in univariate analyses, although not confirmed by multivariate analyses. Therefore, *GSTP1* rs1695 SNP needs to be studied in larger series.

1. Introduction

Cisplatin is an antineoplastic agent which is widely used in the treatment of various adult and childhood malignancies. However it has various important side-effects such as ototoxicity, nephrotoxicity, and neurotoxicity. Incidence of cisplatin ototoxicity has been reported to vary between 13% and 96% in different studies [1–6]. Cisplatin causes high frequency hearing loss, which is usually bilateral and permanent, however hearing thresholds in lower frequencies may also deteriorate with high and cumulative doses.

Cisplatin induces cell death in outer hair cells, stria vascularis, and spiral ganglion cells via intrinsic apoptotic pathway [1–3]. Although many risk factors such as age, gender, cumulative dose, radiotherapy to the head and neck region, co-treatment with other ototoxic drugs have been well described [3,6–8], genetic risk factors of Cisplatin ototoxicity have only recently become of interest and there are only a few studies conducted on children [10–15]. Latest genetic studies mainly focus on single nucleotide polymorphisms (SNP) of genes, coding for enzymes or proteins, such as Megalin (*LRP2*), Glutathione S-transferase (*GST*), Excision Repair

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Cross-Complementing group (*ERCC*), Thiopurine S- Methiltransferase (*TPMT*), and Catechol-O- Methylransferase (*COMT*) [9–14]. Evaluation of genetic and non-genetic factors together was thought to be helpful to understand the mechanisms behind cisplatin ototoxicity. The aim of this study was to investigate the genetic and non genetic risk factors related to cisplatin ototoxicity in pediatric patients.

2. Patients and methods

2.1. Patients and samples

The use of human samples was approved by the Ethics Committee of Dr Behçet Uz Children's Training and Research Hospital (30.11.2012–56) and guided in accordance with Clinical Research guidelines of both Dr. Behcet Uz's Children's Training and Research Hospital and Dokuz Eylul University.

This study was designed as a prospective study. Patients, included in the study received cisplatin chemotherapy in the pediatric oncology departments of Dokuz Eylül University and Dr Behçet Uz Children's Training and Research Hospital between January 2013 and March 2015. After obtaining written informed consent from parents peripheric blood samples were collected from all children.

All patients included in the study received cisplatin chemotherapy for the first time and they were all consecutive patients. Before every cycle of cisplatin chemoterapy hemograms were checked. Cisplatin chemotherapy was administrated to the patients whom hemoglobin concentration was minimum 10gr/dl. All of them were newly diagnosed patients with different malignancies. Patients with a history of hearing loss, noise exposure, any external-middle ear disorders or usage of other ototoxic drugs were not included in the study.

2.2. Audiological assesments

All patients underwent routine otorhinolaryngologic examination and audiologic evaluation before the beginning of cisplatin chemotherapy. Patients hearing thresholds were evaluated by Distortion product otoacoustic emissions (DPOAE) and Auditory brainstem responses (ABR) using a 6 kHz and 8 kHz tone burst stimulus for children under 3 years of age. For older children hearing thresholds were determined by using pure tone audiometry and DPOAE tests. Patients with signs of any external or middle ear disease and/or pre-existing hearing loss were excluded from the study. Audiological assesments were repeated before each cycle of chemotherapy and at a minimum of 3 months after the termination of the cisplatin chemotherapy. Latest audiological findings were used to evaluate the ototoxicity. Brock and Munster classifications were used for these ototoxicity evaluations [15,16].

2.3. Isolation of mononuclear cells from peripheric blood samples

In order to conduct molecular analyses a10 cc blood sample from each patient was collected into tubes with EDTA coagulant. Mononuclear cell isolations were performed by using histopaque. Briefly, the blood samples were diluted with phosphate buffer saline (PBS) in 1:1 ratio. Diluted blood samples were added to 3 ml of histopaque at an angle of 45° to prevent interference of blood samples with the histopaque. The samples were centrifuged for 20 min at 1200 rpm. After centrifugation, mononuclear cells that appeared between the histopaque and the serum, were collected into new tubes and washed with 7 ml of PBS and were centrifuged for 7 min at 1600 rpm. The supernatant was removed and the pellets were resuspended in DMSO containing media (70% incomplete RPMI, 20%FBS, 10%DMSO) and stored at −80 °C until analysis.

2.4. DNA isolation

DNA isolation from mononuclear cells was performed with the 'High Pure PCR Template Preparation kit' (Roche) as described by the manufacturer by the spin column method. DNA samples were stored at −20 °C until analysis.

2.5. Single nucleotide polymorphism analysis with real-time PCR

Single nucleotide polymorphisms for *COMT* rs9332377, *ERCC1* rs11615, *GSTP1* rs1138272, *GSTP1* rs1695, *LRP2* rs2075252, and *TPMT* rs12201199 were identified for each patient with Taq-Man Real-time PCR analysis. PCR reactions were performed by using the corresponding primer/probes (0,2 μM) with LightCycler® FastStart DNA Master HybProbe kit. Analyses were performed using the LightCycler 480 Real-time PCR system (Roche), 5 μl of DNA sample was used per each reaction. DNA was amplified by precycling at 95 °C for 10 min, followed by 50 cycles at 95 °C for 5 s, 56 °C for 10 s and 72 °C for 15 s. Subsequently a melting curve analysis was performed at 95 °C for 20 s, 45 °C for 20 min and 80 °C followed by 1 cycle of cooling at 40 °C for 10 s.

Data analyses were performed with the LightCycler 480 program. 'Tm calling mode' 'Genotyping mode' and differences between Tm (melting temperature) and melting curves were assessed. Samples were evaluated as 'wild-type', 'mutant' or 'heterozygote' according to their Tm values.

2.6. Analyses of non genetic risk factors

Age and gender of the patients, application of high cumulative doses of cisplatin (>400 mg/m^2), radiotherapy to the head and neck region, co-treatment with other ototoxic drugs (Furosemide, carboplatin, aminoglycosides), and bolus/continuous injections were evaluated as non-genetic risk factors.

2.7. Statistical analyses

SPSS 15.0 software (IBM, NY, USA) was used for statistical analyses and $p < 0.05$ was accepted as statistically significant for all analyses. Chi-square and Fischer exact tests were used for the analyses of all genetic and non genetic risk factors according to the Brock and Muenster classifications. Then a logistic regression model was created using the risk factors found to be related with Cisplatin ototoxicity in the univariate analyses. Data distribution to look for outliers in the smaller groups was analysed using the Kolmogrov Smirnov normality test.

Then patients were divided into two subgroups as moderate to severe otoxicity (Grade 2 or higher) and mild or no ototoxicity (Grade 0–1). The same univariate and multivariate analyses were repeated to see if any of these risk factors contributed to the development of moderate to severe ototoxicity.

3. Results

Between January 2013 and March 2015, 76 children with newly diagnosed pediatric malingnancies were treated in those two hospitals. Three patients refused to join the study and one patient was excluded due to previous history of sensorineural hearing loss. Seventy-two of them (40 male, 32 female) agreed to join the study. Mean age of the patients was 10.2 (1–17). The majority of the patients received cisplatin chemotherapy due to neuroblastoma, hepatoblastoma and germ cell tumors (Fig. 1).