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Clinical report

Glutathione S-transferase genetic polymorphisms and individual sensitivity to the ototoxic effect of cisplatin

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One of the side effects of cisplatin therapy in malignant neoplasms is ototoxicity. This effect shows a wide inter-individual range which is more variable than the pharmcokinetic parameters. Oxidative stress has been implicated in cisplatin ototoxicity. The glutathione S-transferase (GST) supergene familiy encodes isoenzymes that appear to be critical in protection against oxidative stress. Certain GST loci are polymophic, demonstrating alleles that are null (GSTM1 and GSTT1), encode low-activity variants (GSTP1) or are associated with variable inducibility (GSTM3). The aim of our study was to investigate genetic risk factors involved in the ototoxicity of cisplatin and to determine whether the polymorphisms in five GST genes affect the individual risk of ototoxicity by cisplatin. Two groups of patients were analyzed in this study: group H, 20 patients early and highly sensitive to the ototoxicity of cisplatin; and group N, 19 patients with no hearing impairment under comparable doses of the drug. We found a protective effect for the GSTM3*B allele with a frequency of 0.18 in the group with normal hearing after therapy versus 0.025 in the group with hearing impairment. (χ^2=5.37; p=0.02). [© 2000 Lippincott Williams & Wilkins.]

Key words: Cisplatin, glutathione S-transferase, ototoxicity, polymorphism.

Introduction

Cisplatin [*cis*-dichlorodiammineplatinum(II)] is a highly effective chemotherapeutic agent but its dose-limiting side effects are nephrotoxicity, peripheral neuropathies and ototoxicity. Nephrotoxicity can be reduced by forced diuresis and by co-administration of nephroprotective drugs, thus giving the other toxicities more clinical prominence. Up to now the biochemical basis for cisplatin toxicity in the cochlea is not known. Histologically, cisplatin causes a degeneration of the outer hair cells of the cochlea with damage progressing from basal to apical. The pattern of hair cell loss explains the pattern of hearing loss.[1] Interindividual differences in sensitivity to hearing loss following cisplatin treatment were observed in clinical studies[2] as well as in animal tests.[3] The reported clinical incidence of cisplatin ototoxicity ranges from 20 to 90%[4,5] depending on the criteria used and the diligence of the search. Although the influence of nutrition,[6] noise stress[7] and high cumulative dose of cisplatin[8] are discussed, no independent risk factors have been identified to date. A recently published study tested the influence of pharmacokinetics on the ototoxic side effect in an animal *in vivo* model.[9] The conclusion drawn was that the inter-individual variability in auditory brainstem response (ABR) threshold shift was far greater than the variability in pharmacokinetics, suggesting that other factors are more important in determining the degree of hearing loss. Our hypothesis is that the individual susceptibility to cisplatin-induced hearing loss is influenced by genetic factors.

Apoptosis is an important process of the inner ear for removal of oxidative stress-damaged sensory cells from the cochlea. Cisplatin ototoxicity initiates the intracellular production of reactive oxygen species and free radicals. The interaction of reactive oxygen species and free radicals with membrane phospholipids of auditory sensory cells creates aldehydic lipid peroxidation products, which function in particular as

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a mediator of apoptosis for auditory neurons and hair cells.[10] All higher organisms have developed a complex variety of mechanisms by which they protect themselves from environmental insult. A number of enzyme superfamilies including gluthathione S-transferases (GSTs) metabolize xenobiotic toxins. The primary function of the cytosolic GSTs is the conjugation of glutathione to electrophilic substrates. However, these enzymes also play an important role in free radical scavenging due to their glutathione-dependent peroxidase activities, which protect the cell from the deleterious effects of oxidative stress. The expression of many of these enzymes is genetically polymorphic and many studies have attempted to relate mutant alleles to disease susceptibility.[11] Increased expression of GST isozymes in tumor cells indicates a role for these proteins in the development of resistance to chemotherapy.

In the rat kidney GSTM activity was increased 1.9-fold after cisplatin administration and the platinum concentration was the highest in the GSTM fraction of renal GST isoenzymes after isolation with affinity chromatography.[12] These authors conclude that GSTM is mainly involved in the metabolism of cisplatin. Some other studies found evidence for an involvement of GSTP in cisplatin resistance. For example, the ratio of GSTP concentration in tumors after cisplatin therapy to that before therapy was significantly higher for nonresponders than for responders.[13,14]

The transfection of GSTP antisense cDNA into cultured human cancer cells, which express an innately high level of GSTP and show intrinsic drug resistance, increased the sensitivity to cisplatin about 3.3-fold.[15] In HeLa cells GSTM3 was the most abundant GST and the concentration of all GSTs was found to be associated with the sensitivity of the cells to cisplatin.[16]

Immunohistochemical analysis showed the expression of GSTP and GSTM in the mammalian inner ear; GSTM are localized in the inner and outer hair cells, and GSTP are confined mainly to Deiters cell processes.[17]

The polymorphisms of the GSTM1 and GSTT1 loci arise from the complete deletion of each gene, and can have a substantial effect on the metabolism of many substrates.[18]

The polymorphisms at the GSTP1 and GSTZ1 loci result in amino acid substitutions, and effect substrate selectivity and stability.[19] The GSTM3*B allele has a 3 base deletion which generates a recognition site for the transcription factor YY1.[20] These polymorphisms and the effects of GSTs on cisplatin metabolism make GSTs plausible candidate genes for susceptibility to ototoxicity. We investigated the association between risk of hearing impairment after cisplatin therapy and polymorphisms in five GST genes.

Subjects and methods

Patients

All 71 probands were children and young adults aged between 3 and 22 years. They were diagnosed between 1991 and 1996 with osteosarcoma (n=44), germ cell tumor (n=4), neuroblastoma (n=18) and brain tumor (n=5). Cisplatin chemotherapy was carried out at Münster University Hospital according to the treatment protocols of the GPOH. Criteria for participation in the study were the informed consent of the patients or their parents, a detailed protocol of the therapy and an audiogram below 10 dB at all frequencies before chemotherapy.

Grading system of hearing loss

All patients underwent hearing assessment before therapy, after every course of cisplatin and at the follow-up checks. They were classified in a grading system based on the results of the audiograms (Table 1). Hearing loss was consistently bilateral.

Table 1. Classification of cisplatin-induced bilateral high-frequency hearing loss[a]

Bilateral hearing loss	Pedaudiological evaluation	Grade
$<$10 dB at all frequencies	no damage	0
Hearing loss 4 kHz $\leqslant$20 dB	no considerable damage	1
Hearing loss 4 kHz $>$20 dB	moderate damage	2
Hearing loss $<$4 kHz	compensable impairment	
$\leqslant$20 dB		3
$>$20–$<$40 dB		3a
$\geqslant$40–$<$60 dB		3b
$\geqslant$60 dB		3c
Deafness	loss of function	4

[a]The results were obtained by pure-tone audiometry from both ears. Additionally, tympanometry and measurement of transient evoked and distortion product emissions were performed. When necessary, brainstem response audiometry was carried out.

Two groups of index patients were selected from the patient collective. Group H comprised those 20 patients suffering early and highly hearing loss but in whom all potentially interfering factors such as cranial radiation, severe renal insufficiency, treatment with other ototoxic drugs and pre-existing hearing loss or familial risk of hearing impairment had been ruled out. The 19-patient control group (group N) had an audiogram without pathologic findings at every registration during and after chemotherapy (Table 2).

Laboratory assays

DNA was isolated from lymphocytes using EDTA blood and standard methods. PCR was performed using Eurogentec Taq polymerase with supplied buffer. A 50 μl reaction contained 200 ng DNA, 1.5 mM $MgCl_2$, 1.0 mM dNTPs, 0.5 μM of each primer and 1 U Taq. PCR segments were digested with restriction endonucleases and the resulting fragments were resolved through electrophoresis in agarose gels visualized by UV-induced fluorescence.

GSTM1 alleles were identified using allele-specific PCR primers from intron 6 and exon 7 to distinguish GSTM1*A and GSTM1*B. GSTM1*0 homozygotes were determined by absence of the specific GSTM1 product with a β-globin fragment co-amplified as internal positive control in the PCR reaction.[21] GSTM3*A and GSTM3*B were identified using specific primers to exon 6/7 and restriction with *Mnl*I.[20] GSTT1*0 homozygotes were determined by absence of the specific GSTT1-product with a β-globin fragment co-amplified as internal positive control in the PCR reaction.[18] GSTP1 gene variants were detected by digestion of specific PCR products with *Alw*26I for polymorphism at nucleotide +313 and *Aci*I for +341; accordingly, GSTP1*A has isoleucine at position 105 and alanine at position 114. GSTP1*B has valine at position 105 , GSTP1*C has valine at positions 105 and 114. GSTZ1 gene variants were detected by digestion of specific PCR products with *Alw*26I for polymorphism Lys32Glu and *Fok*I for Arg42Gly.[19] The GSTZ1*A allele has 32Lys and 42Arg, GSTZ1*B 32Glu and 42Arg, GSTZ1*C 32Glu and 42Gly.

Table 2. Clinical data for the patients

	Group N (n=19)	Group H (n=20)
Mean age at therapy (range, years)	13.6 (7–19)	11.3 (3–22)
Sex (M/F)	10/9	12/8
Histology		
osteosarcoma	12	15
neuroblastoma	1	5
medulloblastoma	3	
germ cell tumor	3	
Grade of hearing loss after therapy 0/2/3	19/0/0	0/8/12
Chemotherapy protocol		
COSS 86/91/96/	1/10/1	1/14/0
Hit 91	3	0
MAKEI 89	3	0
NB-85/90	1/0	0/5
Mean cumulative dose of cisplatin at end of therapy	429	422
Cumulative dose of cisplatin on reaching ⩾grade 2 (mean, range, mg/m^2)		236 (120–357)

Group N: grade 0 and cumultive dose of cisplatin after therapy: ∅429 mg/m^2→19 probands.
Group H: grade⩾2 and cumulative dose of cisplatin at first time registration of grade 2: ∅236 mg/m^2→20 probands.

Statistical methods

The χ^2 test was used for statistical analysis. The level of statistical significance was set at $p<0.05$. Statistical analysis was performed with the JUMBO statistical package (University of Münster, Germany).

Results

The results for the distribution of alleles and genotypes of all five GST genes are presented in Table 3. The 71 cancer patients enrolled in this study were selected with the aim of obtaining an average value for the frequency of various genotypes, because most of the GST polymorphisms are suspected to have distributions differing among cancer cases from those of the overall population.

The most significant polymorphisms with zero alleles for GSTM1 and GSTT1 show no difference between our groups N and H. There is an obvious linkage between the loci GSTM1 and GSTM3 as found by others.[20] Allele GSTM1*A is associated with an increased frequency of GSTM3*B (Table 4). GSTM3*B had a higher allele frequency in group N (0.18) than in the overall population (0.15), but of only 0.025 in group H, representing a significant inter-group difference (N versus H: χ^2=5.37; p=0.02).

The distribution of the genotypes of the GSTP1 polymorphisms differed between the groups. There was a lack of GSTP1*A homozygotes in group N, but this did not correlate with their excess in group H. This difference seemed to be secondary to a higher allele frequency of GSTP1*B in group N (0.56) compared to group H (0.38) and the overall population (0.28), but there was no statistical significance.

Table 3. Distribution of alleles and genotypes of GST polymorphism

Gene	References[a] (%)	Cases (n=71)	Group N (n=19)	Group H (n=20)
GSTM1				
*0	93 (52)	30 (42)	11 (58)	9 (45)
*AA/*A0	57 (32)	28 (40)	5 (26)	6 (30)
*BB/*B0	17 (10)	8 (11)	1 (5)	4 (20)
*AB	11 (6)	5 (7)	2 (10)	1 (5)
GSTM3				
*AA	116 (71)	48 (67)	14 (74)	19 (95)
*AB	47 (28)	19 (27)	3 (16)	1 (5)
*BB	1 (1)	4 (6)	2 (10)	0 (–)
A/B ratio	279/49	115/27	31/7	39/1
	085/0.15	081/0.19	0.82/0.18	0.975/0.025
GSTT1				
*00	47 (23)	16 (23)	3 (16)	8 (40)
*AA/*A0	159 (77)	55 (77)	16 (84)	12 (60)
GSTP1				
*AA	39 (41)	22 (31)	2 (10)	5 (25)
*AB	37 (39)	32 (55)	14 (74)	8 (40)
*BB	7 (7)	8 (11)	2 (10)	4 (20)
*AC	9 (10)	5 (7)	0 (–)	2 (10)
*BC	3 (3)	4 (6)	1 (5)	1 (5)
A/B/C ratio	124/54/12	81/52/9	18/19/1	20/17/3
	0.65/0.28/0.06	0.57/0.36/0.06	0.47/0.5/0.03	0.5/0.42/0.08
GSTZ1				
*AA	47 (49)	34 (48)	11 (58)	10 (50)
*AB	33 (35)	26 (36)	6 (32)	5 (25)
*AC	9 (10)	7 (10)	1 (5)	5 (25)
*BC	4 (4)	2 (3)	1 (5)	0 (–)
*BB	2 (2)	1 (1,5)	0 (–)	0 (–)
*CC	0 (–)	1 (1,5)	0 (–)	0 (–)
A/B/C ratio	136/41/13	101/30/11	29/7/2	30/5/5
	0.72/0.21/0.07	0.71/0.21/0.08	0.76/0.18/0.06	0.75/0.125/0.125

[a]Reference data are taken from Matthias *et al.*[22] for GSTM1, GSTM3 and GSTT1, and from Menegon *et al.*[19] for GSTP1 and GSTZ1.

Table 4. Linkage between the loci GSTM1 and GSTM3

GSTM1	0			A			B			AB		
GSTM3	AA	AB	BB	AA	AB	BB	AA	AB	BB	AA	AB	BB
expected	20	8	2	19	8	1	5	2		4	1	
found	28	2		9	15	4	7	1		4	1	

Discussion

The glutathione transferase M1 is involved in the metabolism of cisplatin and is expressed in the outer hair cells, which are damaged in cases of hearing impairment after cisplatin therapy. Therefore, it seemed plausible that the deletion polymorphism in GSTM1 might have an effect on the individual risk for ototoxicity. In our study, however, we found no association between GSTM1 genotype and risk for ototoxicity by cisplatin nor any correlation with the deletion of GSTT1.

GSTM3 is probably associated with sensitivity to cisplatin in cancer cell lines.[16] In a study searching for risk factors for multiple cutaneous basal cell carcinoma, the genotype GSTM3 AA was found to be associated with increased tumor numbers.[23] The data suggest that GSTM3*B is protective, although the mechanism is unclear. In another study, the GSTM3 AA genotype was associated with a more severe

disease course in MS.[24] The authors suggest that the YY1 transcription factor acts as a GSTM3 inducer via the 5′-AAGATA-3′- motif in GSTM3*B. The enzyme is part of the defence mechanism against oxidative stress and a higher induced expression of the gene provides more antioxidant protection.

Our finding of a lack of GSTM3*B in patients with hearing loss is in agreement with this hypothesis, since oxidative damage is one of the important insults induced by cisplatin cytotoxicity. As the sensory cells of the inner ear are extremely sensitive to effects of reactive oxygen species, it may be possible for individuals with a better genetic condition for antioxidant defense to exhibit a lower sensitivity to the ototoxic effect of cisplatin. We found no evidence that genetic variations in one of the other analyzed GSTs are linked to the predisposition to ototoxicity by cisplatin. The frequency of the GSTM3*B polymorphism, however, is too low to be a major factor regarding the predisposition phenomenon. Finally, our results suggest that the genetic background underlying the individual antioxidant defense mechanism might play a role in this problem. Further investigations of other antioxidant enzymes might help to clarify this question.

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