Relationship between GSTP1 Ile\(^{105}\)Val polymorphism and docetaxel-induced peripheral neuropathy: clinical evidence of a role of oxidative stress in taxane toxicity

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Background: Glutathione-S-transferases (GST) regulate the cellular response to oxidative stress. We previously highlighted the importance of oxidative stress in taxane toxicity and therefore investigated the relationship between the GST isoforms M1, T1 and P1 gene polymorphisms and docetaxel (Taxotere)-induced peripheral neuropathy (DIPN).

Patients and methods: The GSTM1 (null), GSTT1 (null) and GSTP1 (Ile\(^{105}\)Val and Ala\(^{114}\)Val) polymorphisms were determined in a cohort of cancer patients treated with docetaxel and entered in a clinical trial database. The relationship between GST polymorphisms and grade \(\geq 2\) DIPN as primary end point was studied.

Results: Fifty-eight patients (median age 61 years) received a total of 261 cycles of docetaxel given as single agent. Patients with GSTP1\(^{105}\)Ile/\(^{105}\)Ile genotype had a higher risk of developing a grade \(\geq 2\) DIPN than did those with other GSTP1 genotypes (8 of 27 versus 2 of 31, respectively, odds ratio 6.11; 95% confidence interval 1.17–31.94; \(P = 0.03\)). In multivariate analysis, grade \(\geq 2\) DIPN was strongly correlated with GSTP1 Ile\(^{105}\)Ile genotype (\(P = 0.01\)) and the number of cycles (\(P = 0.03\)).

Conclusion: We found a significant correlation between GSTP1 Ile\(^{105}\)Ile genotype and the development of grade \(\geq 2\) DIPN. This finding strongly suggests a role of oxidative stress in the pathophysiology of DIPN.

Key words: docetaxel, GST, oxidative stress, peripheral neuropathy, pharmacogenetics, polymorphism

Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) is a major concern for clinicians, which may preclude the prolonged use of various anticancer agents including taxane, oxaliplatin and most recently epothilones and proteasome inhibitors [1]. Identifying predictive factors for CIPN may determine the choice of an anticancer agent or the optimal number of cycles in patients at high risk for this toxicity.

Docetaxel (Taxotere\(^{\text{TM}},\) Sanofi–Aventis, Paris, France) is a taxoid derivative that displays antitumoral activity alone or in combination against many solid tumors, including breast, lung and prostate cancers [2–4]. Peripheral neuropathy is the major cumulative, limiting toxicity of docetaxel and may occur in up to 37% of patients treated with doses ranging from 60 to 100 mg/m\(^2\) [5–7]. The risk factors for docetaxel-induced neurotoxicity are poorly described. In a recent phase III study [8], grade \(\geq 2\) peripheral neuropathy was observed more frequently in patients who had received a mean cumulative dose of 371 mg/m\(^2\). However, severe forms of docetaxel-induced peripheral neuropathy (DIPN) were also reported after the first cycle of treatment. Diabetic peripheral neuropathy and previous platinum-based treatments are recognized risk factors for taxane-induced neuropathy, as shown in patients receiving paclitaxel [7]. Since DIPN is a cause for dose reduction or treatment discontinuation and may cause significant disability, we aimed to identify patient-dependent parameters for DIPN.

First, we and others demonstrated in vitro that the production of reactive oxygen species (ROS) is a crucial step for taxane cytotoxicity [9, 10]. Secondly, we showed in vivo that taxanes generate production of ROS, and their oxidative effects are modulated by mangafodipir, a superoxide dismutase mimic with catalase and glutathione reductase activities [11]. Our third step was to look for a clinical evidence for the role of oxidative stress in taxane toxicity.

The glutathione-S-transferases (GST) are divided into six major classes (\(\alpha, \mu, \pi, \xi, \zeta\) and \(\omega\)) [12]. These enzymes contribute to the inactivation of various toxic compounds (unsaturated aldehydes, quinines, epoxides and hydroperoxides) formed as secondary metabolites during oxidative stress [13]. Besides, Park et al. [14] reported that expression and catalytic activity of GSTP1 were related to resistance to docetaxel treatment in vitro. Iwao-Koizumi et al.
Polymorphisms in the genes encoding GSTM1 (class μ), GSTP1 (class π) and GSTT1 (class θ) have been associated with enzyme activity variations [16]. Hence, the most common polymorphisms in the GSTM1, GSTP1 and GSTT1 genes can decrease or annihilate the activity of the corresponding enzymes:

- two single-nucleotide substitutions in exon 5 (A313G) and exon 6 (C341T) of the GSTP1 gene lead to Ile<sup>105</sup>Val and Ala<sup>114</sup>Val amino acid substitutions in the catalytic site of GSTP1 and cause a decrease of substrate affinity.
- inherited homozygous deletions of GSTT1 or GSTM1 genes (null genotype) cause a complete absence of enzymatic activity.

Hence, we studied whether the GSTM1, GSTP1 and GSTT1 gene polymorphisms were predictive factors for DIPN in cancer patients treated with docetaxel.

**patients and methods**

**patients**

From November 2002 to June 2004, cancer patients treated with docetaxel in our institution were enrolled in a clinico-pharmacological study aiming to identify predictive factors for docetaxel-induced toxic effects. Previous reports have reported the risk factors for febrile neutropenia in this cohort [17, 18]. All patients signed an informed consent form for blood sample collection to establish the clinical significance of genetic polymorphisms in patients receiving docetaxel and were subsequently included in this retrospective analysis. The protocol was approved by the local ethics committee. Docetaxel (Taxotere<sup>®</sup>, Sanofi-Aventis) was administered through a central i.v. line, as a 60-min infusion. The dose was left to the investigator decision within the standard range of 75–100 mg/m<sup>2</sup>. After first cycle, treatment was continued at the discretion of the treating physician. A total of 261 cycles were administered (median per patient: 4, range 2–12). Patients’ characteristics are presented in Table 1. Fifty-eight patients (sex ratio 1 : 1; 27.6% breast, 27.6% prostate, 24.1% lung and 20.7% other cancers) with a median age of 61 years (range 47–75) were included. Forty-two patients received 85 mg/m<sup>2</sup> docetaxel, eight received 75 mg/m<sup>2</sup>, seven received 60 mg/m<sup>2</sup> and one received 70 mg/m<sup>2</sup>. Ten patients (17.2%) experienced a grade ≥2 DIPN. Univariate analyses were carried out using the Wilcoxon and Fisher’s exact test for continuous and discontinuous variables, respectively. Multivariate logistic regression (with backward and forward procedures) was used to identify the independent factors for grade ≥2 DIPN. All variables with a P value <0.05 in univariate analysis were included in the multivariate models. All statistical tests were two sided, and P < 0.05 was used to indicate statistical significance. Calculations were carried out with NCSS™ software (NCSS 6.0, NCSS, Kaysville, UT).

**DNA extraction and genotyping**

Total genomic DNA was extracted from peripheral lymphocytes using a commercial kit (Qiamp DNA Blood Mini Kit, Qiagen, Courtabœuf, France) and stored at −20°C until analysis. The DNA samples were analyzed using the PCR technique, using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Courtabœuf, France). Previously published methods were used for genotyping GSTM1 and GSTT1 [19] and GSTP1 exons 5 and 6 [20, 21].

**GSTM1 and GST1.** A multiplex PCR was conducted using primer pairs specific to each gene. The fragment lengths of the PCR products were 480 bp for GSTT1 and 215 bp for GSTM1. PCR signals were observed in the presence of the GSTM1 or GSTT1 gene, whereas the deletion generated no signals. The results were validated using the gene encoding β2-
microglobulin as an internal amplification control.

**GSTP1 exon 5.** The PCR product was subjected to restriction fragment analysis using BamAI enzyme (New England Biolabs, Saint Quentin en Yvelines, France). The 176-bp GSTP1-amplified fragment was cut into 91- and 85-bp fragments when the GSTP1<sup>105</sup>Val allele was present and was not cut when the GSTP1<sup>105</sup>Ile allele was present. The amplification products were revealed using an electrophoresis on 1.5% agarose gel and staining with ethidium bromide.

**GSTM1 and GST1.** The amplified DNA was purified using QiaQuick DNA Purification System (Qiagen) and sequenced using BigDye Terminator chemistry and an ABI PRISM 3100 genetic analyzer (Applied Biosystems).

For each genotyping, at least two positive controls were used: one homozygous for the wild allele and one heterozygous, and when it was available one homozygous for the mutated allele. These controls are DNAs that have been sequenced.

**statistical analysis**

The chi-square test was used to compare the observed genotype distributions with those expected by the Hardy–Weinberg equilibrium. GSTM1, GSTP1 and GSTT1 polymorphisms were analyzed separately to evaluate the association between the polymorphisms and DIPN. The primary end point was the development of a grade ≥2 DIPN. Univariate analyses were carried out using the Wilcoxon and Fisher’s exact test for continuous and discontinuous variables, respectively. Multivariate logistic regression (with backward and forward procedures) was used to identify the independent factors for grade ≥2 DIPN. All variables with a P value <0.05 in univariate analysis were included in the multivariate models. All statistical tests were two sided, and P < 0.05 was used to indicate statistical significance. Calculations were carried out with NCSS™ software (NCSS 6.0, NCSS, Kaysville, UT).

**results**

A total of 261 cycles were administered (median per patient: 4, range 2–12). Patients’ characteristics are presented in Table 1. Fifty-eight patients (sex ratio 1 : 1; 27.6% breast, 27.6% prostate, 24.1% lung and 20.7% other cancers) with a median age of 61 years (range 47–75) were included. Forty-two patients received 85 mg/m<sup>2</sup> docetaxel, eight received 75 mg/m<sup>2</sup>, seven received 60 mg/m<sup>2</sup> and one received 70 mg/m<sup>2</sup>. Ten patients (17.2%) experienced a grade ≥2 DIPN (according to the NCI-CTC, version 2.0). Only one patient had diabetes and did not develop DIPN. No difference was noticed between the

<table>
<thead>
<tr>
<th>Primary tumor</th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>16 (27.6)</td>
</tr>
<tr>
<td>Lung (non-small cell)</td>
<td>14 (24.1)</td>
</tr>
<tr>
<td>Prostate</td>
<td>16 (27.6)</td>
</tr>
<tr>
<td>Other</td>
<td>12 (20.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Median (range)</th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>61 (47–75)</td>
<td>28/29 (50/50)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male/female</th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (8.6)</td>
<td>38 (65.5)</td>
<td></td>
</tr>
<tr>
<td>14 (24.1)</td>
<td>1 (1.7)</td>
<td></td>
</tr>
</tbody>
</table>

WHO, World Health Organization.
patients who developed DIPN and the other patients, regarding age, gender or docetaxel dose (Table 2). The genotyping results were available for all patients (Table 3). Patients who exhibited a null genotype for GSTM1 and GSTT1 were 36 of 58 (62.1%) and 17 of 58 (29.3%), respectively. Patients exhibiting GSTP1 Ile/Ile/Val or Ile/Val/Val genotypes were 25 of 58 (43.1%). The above distributions were in close agreement with those predicted by the Hardy–Weinberg equilibrium, and the observed allele frequencies were similar to those previously reported in healthy Caucasian populations [12].

Grade ≥2 DIPN was significantly more common in patients with GSTP1 Ile/Ile/Val genotype (8 of 27 patients, 30%) compared with patients with GSTP1 Ile/Ile/Ile or Val/Val/Val genotypes (2 of 31 patients, 6.5%; Table 4). As a result, patients who were genotyped as GSTP1 Ile/Ile/Ile had a higher risk of developing a grade ≥2 DIPN than did those with other GSTP1 exon 5 genotypes (odds ratio 6.11; 95% confidence interval 1.17–31.94; P = 0.03). No association was found with respect to any of the GSTM1, GSTT1 or GSTP1 exon 6 genotypes.

The multivariate logistic regression (Table 2) found a strong correlation between grade ≥2 DIPN and the following variables: GSTP1 Ile/Ile/Ile genotype and the number of cycles. Previous platinum treatment, age, gender and PS were not associated with grade ≥2 DIPN.

**discussion**

This study found a relationship between the GSTP1 Ile/Ile/Ile polymorphism and the peripheral neurosensory toxicity of docetaxel. Our results indicate that the GSTP1 Ile/Ile/Ile genotype was associated with grade ≥2 DIPN. In multivariate analysis, this genotype was more significantly associated with cumulative neurosensory toxicity than the number of cycles of docetaxel.

Based on our preclinical results indicating a critical role of ROS in taxane toxicity [10, 11], we hypothesized that genes encoding for antioxidant enzymes might influence docetaxel neurosensory toxicity.

The role of GSTP1 in cellular response to oxidative stress is a credible hypothesis to explain the protective effect of the variant allele Ile/Ile. Previous studies [22, 23] revealed that GSTP1 is an inhibitor of the stress-inducible c-Jun NH2-terminal kinase (JNK) and also downregulates the expression of downstream genes of the JNK signaling pathway. The absence of GSTP1 protein in GSTP1 null mice results in increased constitutive JNK activity leading to the upregulation of specific downstream genes implicated in antioxidant cellular response [24]. The GSTP null mice are therefore highly resistant to the hepatotoxic effects of acetaminophen [25]. Therefore, we hypothesize that the patients carrying GSTP1 Ile/Ile/Ile alleles are similarly protected against cumulative peripheral neuropathy to docetaxel. Hence, the GSTP1 Ile/Ile/Ile protein could enhance chemotherapeutic neurotoxicity through inhibition of JNK, whereas the GSTP1 Ile/Ile/Ile protein could allow a higher activity of JNK, inducing the expression of genes involved in cellular defense and thus protecting the cells against chemotherapeutic neurotoxicity.

The mechanism of taxane-induced peripheral neuropathy remains unclear. However, because the survival and function of neurons require that proteins and other components be actively transported along long axons from a neuron’s cell body to its distal synapses, taxanes likely interrupt this active transport since they target β-tubulin, a key component of axonal tubules [7]. However, contradictory results were observed in humans.

**Table 2.** Factors associated with grade ≥2 DIPN: results from multivariate logistic regression analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>P (univariate analysis)</th>
<th>P (multivariate analysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.60</td>
<td>NS</td>
</tr>
<tr>
<td>Gender</td>
<td>0.13</td>
<td>NS</td>
</tr>
<tr>
<td>GSTP1 exon 5 Ile/Ile/Ile</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>No. of cycles</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Dose per cycle</td>
<td>0.26</td>
<td>NS</td>
</tr>
<tr>
<td>PS</td>
<td>0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Previous platinum regimen</td>
<td>0.61</td>
<td>NS</td>
</tr>
</tbody>
</table>

Statistically significant values are given in bold (P < 0.05).

DIPN, docetaxel-induced peripheral neuropathy; NS, not significant; PS, performance status.

**Table 3.** Genotype and haplotype distribution (n = 58)

<table>
<thead>
<tr>
<th>GSTT1 Genotype</th>
<th>n (%)</th>
<th>GSTM1 Genotype</th>
<th>n (%)</th>
<th>GSTP1 Haplotypes</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild or heterozygote</td>
<td>41 (70.7)</td>
<td>Wild or heterozygote</td>
<td>36 (62.1)</td>
<td>105Ile, 114Ala/105Ile, 114Ala</td>
<td>25 (43.1)</td>
</tr>
<tr>
<td>Wild or heterozygote</td>
<td>41 (70.7)</td>
<td>Wild or heterozygote</td>
<td>36 (62.1)</td>
<td>105Ile, 114Ala/105Ile, 114Val, 114Ala</td>
<td>20 (34.5)</td>
</tr>
<tr>
<td>Wild or heterozygote</td>
<td>41 (70.7)</td>
<td>Wild or heterozygote</td>
<td>36 (62.1)</td>
<td>105Val, 114Ala/105Val, 114Ala</td>
<td>7 (12.0)</td>
</tr>
<tr>
<td>Wild or heterozygote</td>
<td>41 (70.7)</td>
<td>Wild or heterozygote</td>
<td>36 (62.1)</td>
<td>105Val, 114Ala/105Val, 114Val</td>
<td>4 (6.9)</td>
</tr>
<tr>
<td>Wild or heterozygote</td>
<td>41 (70.7)</td>
<td>Wild or heterozygote</td>
<td>36 (62.1)</td>
<td>115Val, 114Ala/115Val, 114Val</td>
<td>2 (3.5)</td>
</tr>
</tbody>
</table>

**Table 4.** Genotype distribution according to the primary judgment criteria (occurrence of grade ≥2 DIPN)

<table>
<thead>
<tr>
<th>GSTT1 Genotype</th>
<th>n (%)</th>
<th>GSTM1 Genotype</th>
<th>n (%)</th>
<th>GSTP1 Haplotypes</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild or heterozygote</td>
<td>41 (70.7)</td>
<td>Wild or heterozygote</td>
<td>36 (62.1)</td>
<td>105Ile, 114Ala/105Ile, 114Ala</td>
<td>25 (43.1)</td>
</tr>
</tbody>
</table>

Statistically significant values are given in bold (P < 0.05).

DIPN, docetaxel-induced peripheral neuropathy.
In particular, microtubule aggregation was not observed in sural nerve biopsies from patients with taxane-induced neuropathy [26]. Finally, a recent study highlighted that docetaxel neurotoxicity not only involves the microtubular system of peripheral nerves but also other cellular targets, especially the dorsal root ganglia [26]. Hence, a contribution of oxidative stress to the pathophysiology of DIPN seems a plausible hypothesis.

A significant correlation between the GSTP1 Ile105Val polymorphism and the occurrence of oxaliplatin-induced cumulative neuropathy was recently described [27]. Previous studies demonstrated that the Ile105Val polymorphism decreases the substrate affinity of the GSTP1 enzyme [28, 29]. Hence, individuals homozygous for the GSTP1 Ile105Val genotype had an altered activity depending on substrates compared with individuals homozygous for the GSTP1 105Ile/105Ile allele [30]. Thus, the increased rate of oxaliplatin-induced peripheral neuropathy in patients carrying the GSTP1 105Ile/105Ile genotype could not be explained by a diminished capacity of detoxifying oxaliplatin, given that the enzyme encoded by the allele 105Ile is more active than the allele 105Val against platinum derivatives. Moreover, since GSTP1 does not contribute to the clearance of docetaxel, this mechanism could not account for the correlation between GSTP1 polymorphisms and DIPN we found in this study.

Moreover, these results question the potential relationship between GSTP1 polymorphisms and the neurosensory toxicity of other anticancer drugs, especially epothilones and paclitaxel. Sissung et al. [31] reported a relationship between paclitaxel-induced neurotoxicity and ABCB1 polymorphisms in a cohort of 26 patients. The same authors also described a relationship between paclitaxel-induced neurotoxicity and paclitaxel pharmacokinetics. However, to date, no study investigated the clinical relevance of GST polymorphisms in patients treated with paclitaxel.

Finally, the interest of antioxidants for the prevention or the treatment of DIPN remains to be defined. We suggest that glutathione, which has recently demonstrated promising clinical activity for the prevention of oxaliplatin-induced neurotoxicity [32], could be evaluated in patients receiving docetaxel.

In conclusion, the occurrence of grade 2/2 DIPN was significantly more frequent in patients homozygous for GSTP1 105Ile allele in this study. This finding strongly suggests a role of oxidative stress in DIPN pathophysiology and opens new avenues to explore, in order to better understand and treat taxane neurotoxicity.

**acknowledgement**

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**references**


