RESEARCH ARTICLE

GSTT1 null and *MPO -463G>A* Polymorphisms and Carboplatin Toxicity in an Indian Population

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Abstract

Carboplatin, a second generation platinum drug, is widely used to treat different types of cancers. However, myelosuppression remains a major consideration in its use. Genetic polymorphisms of enzymes involved in drug disposition can influence therapeutic outcome. The homozygous null deletion of phase II metabolic gene GSTT1 that abolishes its xenobiotic- detoxifying ability may be associated with carboplatin toxicity. Further, since carboplatin generates oxidative stress, polymorphisms of oxidative stress genes that regulate the cellular level of free radicals may have important roles in generating drug- related adverse effects. We here investigated the null polymorphism of GSTT1, and the -463G>A promoter polymorphism of oxidative stress gene myeloperoxidase (*MPO*) for carboplatin toxicity in a population of northern India. Cancer patients who were treated with carboplatin, and developed toxicity was considered. The study group comprised of 10 patients who developed therapy- related adverse effects. Peripheral blood was taken from patients for DNA isolation. *GSTT1* null genotype was determined by conducting duplex PCR and *MPO-463 G>A* was determined by PCR followed by RFLP. Hematologic toxicity was experienced by 5 patients, 2 of them had grade 3 and 4 toxicity and 3 others had grade 2 toxicity. They also had gastrointestinal (GI) toxicity. Remaining 5 individuals developed GI toxicity but no hematological toxicity. While GG homozygous of *MPO* was present in majority of patients having hematologic toxicity (in 4 out of 5 individuals), one A allele (AG genotype) was present in 4 patients who did not have any hematological toxicity. Thus variant *A* allele of *MPO -463G>A* may be related to lower hematological toxicity. These preliminary data, however, are required to be confirmed in larger studies along with other relevant polymorphisms.

Keywords: Carboplatin - *GSTT1* - kumaun region - *MPO* - polymorphism - toxicity

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Introduction

Platinum drugs are most commonly used in present day cancer chemotherapy. However, severe drug- related toxicity which is often irreversible remains major limitation in their use. Carboplatin, an analogue of cisplatin has antitumour activity similar to cisplatin but different spectra of toxicity. It causes less nephrotoxicity, neurotoxicity, ototoxicity and gastrointestinal toxicity than cisplatin. However, myelosuppression, which is uncommon with cisplatin, is the dose- limiting toxicity of carboplatin (O'dwyer et al., 1997).

Among many potential causes of individual variability for drug response, genetic variation has been recognized as an important determining factor for more than five decades (Ma and Lu, 2011). Genetic alterations in drug-metabolizing enzymes may cause such variability in drug-related adverse effects (Donnelly, 2004; Nakamura, 2008). Glutathione S-transferases (GSTs) constitute a multigene family of metabolizing enzymes and are involved in phase II cellular detoxification of reactive metabolites by conjugating glutathione to electrophilic functional groups and rendering them more water soluble (Hayes and Pulford, 1995). *GSTT1* gene is a member of GST theta class, the most ancient class among seven classes of GSTs (Di Pietro et al., 2010). A null polymorphism or total gene deletion (-/-) which abolishes gene activity is found for *GSTT1*. Since *GSTT1* is constitutively expressed in the liver (Thorn et al., 2012) and is involved in detoxification process of platinum drugs (Moyer et al., 2010) the null genotype may have an important role in generating therapeutic outcome.

Further, carboplatin generates oxidative stress (Cheng et al., 2008; Lin et al., 2010) which can lead to toxic effects (Husain et al., 2001). Therefore, genetic polymorphisms of enzymes related to cellular oxidative stress may lead to

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adverse events in cancer patients. It has been found that platinum drugs like carboplatin are regulated by genes involved in DNA detoxification, e.g., myeloperoxidase (Marsh et al., 2007). Myeloperoxidase *MPO*, a lysosomal enzyme found abundantly in neutrophils, generates reactive oxygen species (ROS) that can be released outside the cell and have potential to damage extracellular target (Klebanoff, 1999). A single nucleotide polymorphism, *-463G>A* at its promoter site leads to loss of SP1 transcription factor binding site (Piedrafita et al., 1996) and subsequent reduced production of ROS. Thus A allele of *MPO* can be hypothesized to have a protective role from carboplatin- toxicity.

In the present study we investigated *GSTT1* null (-/-) and *MPO-463G>A* polymorphisms in cancer patients treated with carboplatin and developing toxicity.

Materials and Methods

Selection of patients

Cancer patients taking treatment with carboplatin at Swami Ram Cancer Hospital and Research Center, Haldwani were recruited as study subjects. This is a referral hospital which caters people from hills and planes of Kumaun region of northern India and adjoining areas of neighboring states (Bag et al., 2012). Four hundred fifty milligram of carboplatin was given as standard dose to them either in single or combined regimen. Signed informed consent was collected from each study participant. Ethical approval for the study was obtained from Institutional Ethical Committee, Govt. Medical College, Haldwani. Toxicity was scored according to the NCI.CTC common toxicity criteria version 2.0. Information on toxicity was collected by researcher unaware of the genotypes of patients.

DNA extraction

Two to five millilitre of peripheral blood was collected in sterile EDTA tube. DNA was extracted from whole blood following standard phenol- chloroform method (Sambrook et al., 1989).

Detecion of GSTT1 (-/-)

Homozygous gene deletion polymorphism (-/-) for *GSTT1* was detected by performing duplex polymerase chain reaction (PCR) as described earlier (Bag et al., 2013). Primer set for *GSTT1* genotypes was within the *GSTT1* gene and another set of primer was selected from human mitochondrial manganese superoxide dismutase (*MnSOD*) as positive control of PCR. Absence of a 131bp band indicated homozygous deletion (null; -/-) for *GSTT1*. Presence of this band indicated homozygous or heterozygous genotypes (+/+ or +/-). This protocol did not differentiate between genotypes either with one or both copies of the gene. A 175 bp band of positive control (*MnSOD*) indicated successful PCR. Duplex PCR were repeated for all samples showing null genotype.

Detection of -463G>A of MPO

PCR was carried out using 10 pmol of each forward and reverse primer, 5'-GGTATAGGCACACAATGGTGAG-3' and 5'-GCAATGGTTCAAGCGATTCTTC-3', respectively (Eurofins, Bangalore). PCR master mix (Promega) was used. PCR condition followed an initial denaturation at 94^{0}C for 2 mins followed by 30 cycles of 30 sec denaturation at 94^{0}C, 30 sec annealing at 58^{0}C, 30 sec extension at 72^{0}C, and final extension at 72^{0}C for 5 mins. Each set of reaction had negative control.

SNP of *MPO* was detected by digesting 6 μl of PCR product with 0.5U of AciI restriction enzyme (New England Biolab) at 37°C for 1½ hr. Digestion product was visualized in polyacrylamide gel. Three bands at 169,120 and 61 were produced for G/G homozygotes, two bands at 289 and 61 bp were produced for A/A homozygotes and heterozygotes (G/A) produced four bands at 289, 169, 120 and 61 bp. Randomly chosen 5 samples were sequenced (SciGenome, India) for confirmation of the restriction digestion results and the reproducibility was 100%.

Table 1. Carboplatin Toxicity and Genetic Polymorphisms in Cancer Patients

Patient	Gastrointestinal toxicity	Hematologic toxicity	Nephro toxicity	*GSTT1* genotype	*MPO* genotype
1	-	4	4	null	GG
2	3	3	-	present	GG
3	2	2	-	present	GG
4	1	2	-	null	AG
5	-	2	-	present	GG
6	3	-	-	present	AG
7	1	-	-	present	GG
8	2	-	-	present	AG
9	1	-	-	null	AG
10	-	-	1	present	AG

Results

During this study period from Dec, 2011 to Nov., 2012 total 33 patients were identified who were treated with carboplatin. We were able to note toxicity status for 10 patients which is presented in Table 1. Patient 1 to 5 had hematological toxicity ranging from grade 2 to grade 4. Patients 6 to 10 did not have hematological toxicity although had other types of toxicities. Out of 5 patients with hematological toxicity, 4 were homozygous for G allele at -463 position of *MPO*. While in the patient group without hematological toxicity, majority (80%) had AG alleles. This indicates that A allele of *MPO -463G>A* polymorphism may be related to reduced hematological toxicity. *GSTT1* genotype did not show any relation with a particular type of toxicity. However, in patient 1 *GSTT1* null genotype in combination with risky genotype of *MPO* may have contributed in generating severe hematologic toxicity, and also renal toxicity which is less frequently found for carboplatin.

Discussion

The second- generation platinum drug carboplatin is widely used to treat lung, ovarian, head and neck cancer (Lin et al., 2010). However, myelosuppression often limits its use. In this study we noted grade 4 and 3 hematologic toxicity in two patients and grade 2 toxicity in 3 of them

while other 5 patients did not have this toxicity.

It is known that genetic factors potentially influence the pharmacokinetics of anticancer drugs (Undevia et al., 2005). Thus polymorphisms in drug metabolizing enzyme like *GSTT1* may be a putative influencing factor in therapeutic outcome. *GSTT1* has been found to be a probable factor for therapy- related adverse events in medulloblastoma (Barahmani et al., 2009). However, in a study on malignant mesothelioma patients treated with either cisplatin or carboplatin combined regimen, *GSTT1* null was found to have no influence on treatment- related leucopenia or thrombocytopenia (Erculj et al., 2012). In this study no role for *GSTT1* in producing hematologic toxicity or other carboplatin- related toxicity was observed.

Myeloperoxidase *MPO* catalyzes the formation of strong oxidant hypochlorous acid (HOCl) from hydrogen peroxide (H_2O_2). In normal condition HOCl is required for defense against microbial infection. However, its overproduction may have potential to cause unwanted damage to normal cells. In vitro experiment revealed that carboplatin generates H_2O_2 (Palma and Aggarwal, 1994) that helps in its tumoricidal activity. But at the same time it increases cellular H_2O_2 pool that may lead to enhanced production of HOCl. In addition, both H_2O_2 and HOCl function as inflammatory mediators (Ochoa et al., 1997). Therefore, they may lead to carboplatin – related toxicity. Although *MPO-463G>A* promoter polymorphism has been studied for risk of different types of cancers (He et al., 2009), it has rarely been studied for predisposition to platinum drug related toxicity. In the present study AG genotype was found in the patients without hematologic toxicity indicating a plausible relation with reduced hematological toxicity.

To the best of our knowledge, association between these two genetic polymorphisms and carboplatin-toxicity has been studied for the first time in an Indian population. Although this was a preliminary study with small number of individuals studied. Larger studies are required to demonstrate actual role of *GSTT1* null and *MPO -463G>A* polymorphisms with carboplatin- toxicity. It has been found that treatment outcome can vary among ethnicities (Millward et al., 2003; Soo et al., 2012), and is an important factor to be considered during assessment of drug efficacy. Therefore, more experiments in this population of northern India can be carried out which may reveal potential differences in carboplatin- treatment outcome between this and other populations. Further, not only *MPO* but also other enzymes that balance cellular free radical levels by neutralizing them, can be studied for carboplatin toxicity.

In conclusion, the present study indicates a probable link between variant morph of *MPO*-463 G>A and reduced carboplatin- related hematological toxicity in a north Indian population, which requires larger study including other polymorphisms in oxidative stress pathway for a better comprehensive understanding.

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