From www.bloodjournal.org by on January 28, 2011. For personal use only.

blood

2004 104: 1574-1577
Prepublished online May 13, 2004;
doi:10.1182/blood-2003-11-3778

Glutathione S-transferase M1 polymorphism: a risk factor for hepatic venoocclusive disease in bone marrow transplantation

Alok Srivastava, Balasubramanian Poonkuzhali, Ramachandran V. Shaji, Biju George, Vikram Mathews, Mammen Chandy and Rajagopal Krishnamoorthy

Updated information and services can be found at:
http://bloodjournal.hematologylibrary.org/cgi/content/full/104/5/1574

Articles on similar topics may be found in the following *Blood* collections:
Transplantation (1589 articles)
Clinical Trials and Observations (3053 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://bloodjournal.hematologylibrary.org/misc/rights.dtl#repub_requests

Information about ordering reprints may be found online at:
http://bloodjournal.hematologylibrary.org/misc/rights.dtl#reprints

Information about subscriptions and ASH membership may be found online at:
http://bloodjournal.hematologylibrary.org/subscriptions/index.dtl

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.
Copyright 2011 by The American Society of Hematology; all rights reserved.

From www.bloodjournal.org by on January 28, 2011. For personal use only.

Glutathione *S*-transferase M1 polymorphism: a risk factor for hepatic venoocclusive disease in bone marrow transplantation

Alok Srivastava, Balasubramanian Poonkuzhali, Ramachandran V. Shaji, Biju George, Vikram Mathews, Mammen Chandy, and Rajagopal Krishnamoorthy

Hepatic venoocclusive disease (HVOD) in bone marrow transplantation (BMT) is attributed to toxicity of cytoreductive agents, especially busulfan and cyclophosphamide, in the conditioning therapy. Busulfan, as well as the metabolites of cyclophosphamide, are conjugated with glutathione (GSH), catalyzed by enzymes of the glutathione *S*-transferase (GST) family. To assess the impact of polymorphisms of the GST genes, *GSTM1* and *GSTT1*, on the risk of HVOD, we evaluated 114 consecutive patients with β-thalassemia major undergoing BMT. There was a significantly increased incidence of HVOD in patients with the *GSTM1*-null genotype compared with those with the *GSTM1*-positive genotype (46.5% vs 18.3%; *P* = .001). Pharmacokinetic analysis in these patients showed that the clearance of busulfan was higher and first-dose steady-state concentration was lower among those with HVOD (0.403 ± 0.06 vs 0.33 ± 0.071 L/h/kg, Student *t* test *P* value = .000 01; and 508 ± 125 vs 656 ± 255 ng/mL, *t* test *P* value = .001, respectively). We conclude that the *GSTM1*-null genotype predisposes to HVOD, and the sinusoidal endothelial cells and hepatocyte damage may be mediated by metabolites of busulfan through depletion of the cellular GSH pool. (Blood. 2004;104: 1574-1577)

© 2004 by The American Society of Hematology

Introduction

Hepatic venoocclusive disease (HVOD) in patients undergoing bone marrow transplantation (BMT) results from conditioning therapy–related damage to the sinusoidal endothelial cells (SECs) and the surrounding centrilobular hepatocytes,[1,2] contributing to significant treatment-related morbidity and mortality.[3] The pathogenesis of HVOD is not fully understood. Although age, sex, cytomegalovirus (CMV) infection, and type of transplant[4,5] have been associated with this complication, cytotoxic drugs used in the conditioning regimen are considered to be the most significant causal factors for the development of HVOD.[6,7] Low glutathione (GSH) levels in hepatocytes (intrinsic or drug-induced) may contribute to such cellular damage.[8] Plasma levels of the cytoreductive agents commonly used in the conditioning regimen, busulfan (Bu) and cyclophosphamide (Cy) and/or its metabolites, have been associated with increased risk of HVOD.[9,10] The association between elevated Bu levels and HVOD has been attributed to Bu-mediated depletion of hepatic GSH, which in turn predisposes hepatocytes to injury from ensuing Cy exposure.[11] Bu is metabolized by the liver cytosolic glutathione *S*-transferases (GSTs) to form a positively charged sulfonium ion that is cleaved to a lipophilic compound, tetrahydrothiophene, which is also toxic to SECs and hepatocytes.[12] Whether Bu itself, the sulfonium ion, or other metabolites are responsible for the toxicity is unclear. GSTA1-1 is the predominant isoform of GST catalyzing the conjugation of Bu with GSH, whereas GSTM1-1 and GSTP1-1 contribute 46% and 18% of the activity of GSTA1-1.[13,14] Of the 4 main subfamilies of GST (A1, M1, T1, and P1), GSTM1 and GSTT1 forms are known to be highly polymorphic with homozygous deletion of either or both genes at varying but significant frequencies in different ethnic groups.[15,16] Cy is not directly toxic to the SECs[17]; however, when metabolized by hepatic cytochrome P450 enzymes, its metabolite, cyclophosphamide mustard, which is a substrate for GSTA1-1,[12] causes toxicity to the SECs.

Patients with thalassemia major undergoing BMT are particularly prone to developing HVOD and the incidence can be as high as 30% to 40%[18,19] in those with significant pre-existing hepatic damage. The aim of the present study was to assess whether the polymorphisms of the GST genes are associated with the risk of developing HVOD in this group of patients.

Patients, materials, and methods

Patients

All patients with β-thalassemia major undergoing BMT at our center from the year 1995 to 2002 were included in the analysis. Patients were randomized to receive total Bu dose of 16 mg/kg or 600 mg/m^2 in 4 divided doses every day on days −9 to −6 and 50 mg/kg Cy intravenously once daily on days −5 to −2. Antithymocyte globulin (30 mg/kg; Lymphoglobuline; Pasteur Meriuex, Paris, France) was given on days −4 to −2, only to those receiving Bu at 16 mg/kg. All patients received 7.5 mg/kg phenytoin orally in divided doses as prophylaxis against seizures, starting one day before and stopping one day after Bu treatment. Informed consent was obtained from the parents of all patients. The study was approved by the Institutional Review Board of Christian Medical College (Vellore, India).

From the Department of Haematology, Christian Medical College, Vellore, India; and Institut National de la Santé et de la Recherche Médicale (INSERM) U 458, Hopital Robert Debre, Paris, France.

Submitted November 6, 2003; accepted April 29, 2004. Prepublished online as *Blood* First Edition Paper, May 13, 2004; DOI 10.1182/blood-2003-11-3778.

Supported in part by the Indo-French Center for the Promotion of Advanced Research (IFCPAR), project no. 2403-2.

A.S. and B.P. contributed equally to this study.

Reprints: Alok Srivastava, Department of Haematology, Christian Medical College, Vellore 632 004, India; e-mail: aloks@cmcvellore.ac.in.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2004 by The American Society of Hematology

From www.bloodjournal.org by on January 28, 2011. For personal use only.

Table 1. Comparison of weight gain and bilirubin levels after BMT in patients with and without HVOD

	Maximum weight gain, % (range)	Maximum bilirubin level, μM (range)
With HVOD	14.24 ± 4.95 (6.3-27.5)	116.96 ± 116.45 (37.62-506.16)
Without HVOD	4.67 ± 3.8 (0-9.3)	21.03 ± 9.41 (8.55-30.78)
P	.00001	0.00001

The maximum weight gain and the highest bilirubin level in the first 20 days after BMT are shown.

The conversion factor for serum bilirubin level is as follows: mg/dL × 17.1 = μM.

Diagnosis of HVOD and analysis of potential contributing factors

HVOD was diagnosed based on Baltimore criteria[20] as follows: development of hyperbilirubinemia with serum bilirubin level greater than 34.2 μM (2 mg/dL) with any 2 of the symptoms including ascites, painful hepatomegaly, and unexplained weight gain of more than 5% from baseline within 20 days of BMT. Liver biopsies were not done for diagnosis of HVOD but the liver was examined at autopsy in 2 patients who succumbed to HVOD. Weight was recorded twice a day and liver function tests were assessed at least twice a week in all patients. Age, serum bilirubin level, ferritin level, aspartate aminotransferase (AST) level, alanine aminotransferase (ALT) level, hepatitis B or C virus infection, and Lucarelli et al's[21] classification of patients were correlated with the incidence of HVOD.

Busulfan assay and pharmacokinetic analysis

Bu levels in plasma samples were analyzed by a high-performance liquid chromatography (HPLC) method using derivatization with tetrafluorothiophenol as previously reported.[22] Pharmacokinetic analysis was done using the TOPFIT program (Gustav Fischer, Stuttgart, Germany)[23] as described previously.[18] Bu doses were not adjusted to achieve any predetermined target level.

Assay of hepatic GSH

Glutathione levels in pre-BMT liver biopsy tissue homogenates were measured by HPLC with fluorescent detection as described previously.[24] Glutathione levels are expressed as nmol/mg protein.

Analysis of the GSTM1/T1 polymorphism

Genomic DNA from peripheral blood from all patients collected before BMT and from 250 healthy controls was subjected to a multiplex polymerase chain reaction (PCR) procedure reported earlier[25] for simultaneous amplification of the *GSTM1* and *GSTT1* genes using albumin as the internal control. The genotype assignment was made by electrophoretic analysis of the amplified product in 2% agarose gel. This technique clearly identifies the homozygous null genotype but does not discriminate the deletional heterozygotes from nondeletional homozygotes, both of which were classified as GSTM1-positive genotype.

Statistical analysis

Statistical significance of the difference between groups was calculated by chi-square test, Fischer exact test, or Student *t* test as applicable. Crude odds ratio (OR) was calculated with 95% confidence intervals. All statistical analyses were performed using Epi Info statistical program (Centers for Disease Control and Prevention, Atlanta, GA)[26] and SPSS version 7.5 (Chicago, IL) for Windows. Multivariate logistic regression analysis was done for the possible predictors of HVOD as independent variables.

Results

A total of 114 patients with β-thalassemia major underwent BMT during the period of this study. Their median age was 6 years (range, 2-16 years). Pretransplantation risk stratification by Lucarelli et al's[21] classification placed 9 patients in class I, 49 in class II, and 56 in class III. The maximum weight gain and the highest bilirubin level in the first 20 days after BMT are shown in Table 1. All patients with HVOD had hepatomegaly more than 2 cm.

The maximum bilirubin level and the percentage weight gain before day +20 was significantly higher in patients with HVOD than those without HVOD (116.96 ± 77.29 μM vs 20.86 ± 8.55 μM [6.84 ± 4.52 mg/dL vs 1.22 ± 0.5 mg/dL]; $P = .00001$ and 14.2% ± 4.95% vs 3.6% ± 2.1%; $P = .00001$, respectively), and this was also the case between those patients with GSTM1-null and GSTM1-positive genotypes (bilirubin level 109.44 ± 71.82 μM vs 37.62 ± 34.20 μM [6.4 ± 4.2 mg/dL vs 2.2 ± 2 mg/dL]; $P = .03$; weight gain 9.5% ± 7% vs 4.2% ± 4%; $P = .01$, respectively). Pretransplantation bilirubin levels were similar between these 2 groups. There was a significantly higher incidence of HVOD in patients with the GSTM1-null genotype compared with those with the GSTM1-positive genotype (46.5% vs 18.3%; $P = .001$; Table 2). There was no significant difference in the incidence of HVOD in patients with GSTT1-null or -positive genotype (18% vs 33.5%; $P = .13$).

Of the 33 patients with HVOD, 8 had severe disease. Seven of them died with HVOD as the major cause of death. Six (75%) of 8 of these patients had the GSTM1-null genotype. The GSTT1-null genotype was seen in 6 (18%) of 33 patients with HVOD against 28 (35%) of 81 patients without HVOD.

The mean hepatic GSH levels in the pretransplantation liver biopsy homogenates of 58 patients for whom the data were available showed no significant difference between those with or without HVOD (48.7 ± 36 vs 40 ± 35 nmol/mg protein; $P = .38$, respectively).

The results of univariate and multivariate analysis of the known risk factors of HVOD are given in Table 3. Of all the variables that appeared significant on univariate analysis, only age and GSTM1 genotype turned out to be significant predictors of HVOD on multivariate analysis.

The clearance (Cl/F) and steady-state concentration (Css) of oral Bu after the first dose was significantly higher in thalassemic patients who developed HVOD compared with those who did not develop HVOD (0.403 ± 0.06 vs 0.33 ± 0.071 L/h/kg; $P = .00001$ and 508 ± 125 vs 656 ± 255 ng/mL; $P = .001$, respectively). When Bu Cl/F was included as one of the variables in multivariate analysis, patients with a Cl/F above 0.37 L/h/Kg had a significantly higher risk of developing HVOD compared with those with a Cl/F below 0.37 L/h/Kg (OR, 4.1; $P = .02$). Further, patients with the GSTM1-null genotype showed significantly higher Cl/F and lower Css of Bu after the first dose compared with those with the GSTM1-positive genotype (Table 4).

Table 2. Frequency of GSTM1 and GSTT1 genotypes in patients with and without HVOD

	GSTM1 null, n (%)	GSTM1 positive, n (%)	GSTT1 null, n (%)	GSTT1 positive, n (%)
$HVOD^-$	23 (53.5)	58 (81.7)	28 (82)	53 (66.5)
$HVOD^+$	20 (46.5)*	13 (18.3)*	6 (18)†	27 (33.5)†

Numbers in each group are as follows: GSTM1 null, n = 43; GSTM1 positive, n = 71; GSTT1 null, n = 34; and GSTT1 positive, n = 80.

*$P = .001$ by chi-square test.

†$P = .13$ by chi-square test.

From www.bloodjournal.org by on January 28, 2011. For personal use only.

Table 3. Pre-BMT characteristics of patients and their influence on the incidence of HVOD

			Univariate analysis			Multivariate analysis		
	With HVOD	No HVOD	OR	95% CI	*P*	OR	95% CI	*P*
Age, y, mean ± SD	9.58 ± 4.6	6.52 ± 3.8	0.844	0.76-0.94	.0014	0.832	0.71-0.98	.02
Serum bilirubin level, μM, mean ± SD	20.18 ± 11.97	15.05 ± 7.7	0.38	0.179-0.803	.0112	0.488	0.201-1.18	.112
Serum ferritin, ng/mL, mean ± SD	3799 ± 1728	3400 ± 2095	0.999	0.99-1.0	.334	1.002	0.99-1.0	0.528
AST, U/L, mean ± SD	98 ± 74	61 ± 28	0.984	0.97-0.99	.0014	0.988	0.97-1.0	.143
ALT, U/L, mean ± SD	132 ± 102	77 ± 56	0.99	0.98-0.99	.0016	0.997	0.988-1.0	.52
Lucarelli, n								
Class I	2	7	1.0	NA	NA	1.0	NA	NA
Class II	10	39	2.1	0.39-11	.38	2.246	0.28-17.6	.441
Class III	21	35	2.34	0.97-5.6	.058	2.062	0.22-19.54	.528
Hepatitis B/C infection, n	8	4	6.16	1.7-22.2	.0055	1.583	0.23-10.7	.34
GSTM1-null genotype, n	20	23	4.12	1.76-9.67	.0011	4.29	1.46-12.53	.0078
GSTT1-null genotype, n	6	28	0.421	0.155-1.14	.0884	0.563	0.163-1.94	.362

The conversion factor for serum bilirubin level is as follows: mg/dL × 17.1 = μM.
NA indicates not applicable.

Discussion

HVOD is a frequent complication of hematopoietic stem cell transplantation,[1,5] occurring at a frequency as high as 45% in those with pre-existing liver dysfunction, as in patients with thalassemia major.[18,19] Various pretransplantation and transplantation-related risk factors have been implicated in the pathogenesis of HVOD. However, no single factor has so far been shown to be independently significant. Our study in a large uniform cohort of patients with β-thalassemia major undergoing allogeneic BMT shows that the incidence of HVOD is significantly higher in patients with the GSTM1-null genotype compared with those with the GSTM1-positive genotype.

The 2 main cytoreductive agents used in the conditioning therapy for BMT, Bu and Cy, involve GST and GSH in their metabolic pathway. The major route of detoxification of Bu is by conjugation with GSH in the presence of GSTs and oxidation of the resulting lipophilic metabolite, tetrahydrothiophene (THT), by cytochrome P450 enzymes before excretion. On the other hand, Cy is first biotransformed by the cytochrome P450 system to form the active metabolite 4-hydroxy Cy, which requires GSH and GST for further metabolism,[11] making GST/GSH pool as a metabolic link between these 2 drugs. This is consistent with the previous findings that prior administration of Bu significantly altered exposure to Cy.[27] The observed increase in the incidence of HVOD in the GSTM1-null patients may be due to altered metabolism of these drugs, generating toxic metabolites that result in increased risk of HVOD.

Multivariate logistic regression analysis of all the potential risk factors of HVOD (age, serum bilirubin level, ferritin level, AST level, ALT level, hepatitis B and C virus infections, and Lucarelli et al's classification of patients) revealed age and the GSTM1-null genotype as the only significant predictors of HVOD (OR, 0.832; $P = .02$; OR, 4.12; $P = .0011$). Age has previously been shown to be a risk factor.[3,4] Our data highlights the importance of GSTM1-null status as a new and additional risk factor for HVOD.

Table 4. Busulfan pharmacokinetic parameters with reference to GSTM1 genotype

	Css-1, ng/mL	Cl/F-1, L/h/kg
GSTM1 null	544 ± 184	0.40 ± 0.064
GSTM1 positive	667 ± 256	0.333 ± 0.071
*P**	.001	.00001

Css-1 indicates steady-state concentration after the first dose of busulfan; and Cl/F-1, clearance after the first dose of busulfan.
**P* value calculated by 2-tailed *t* test.

To further explore the potential mechanisms that could explain the association of the GSTM1 polymorphism with the elevated risk of developing HVOD, we examined the correlation between Bu pharmacokinetic parameters and HVOD. We found that the Cl/F of oral Bu was significantly higher in thalassemic patients who developed HVOD compared with those who did not develop HVOD. Patients with Cl/F above 0.37 L/h/kg had a significantly higher risk of developing HVOD compared with those below this level (OR, 4.1; $P = .02$). Patients with the GSTM1-null genotype showed significantly higher Cl/F and lower first-dose Css of Bu compared with those with the GSTM1-positive genotype (Table 4). This is also consistent with our earlier observation of significantly increased incidence of HVOD in patients with lower Bu area under the concentration versus time curve (AUC) and hence higher Bu Cl/F.[18] Based on these data, it is likely that HVOD results from hepatocyte/SEC damage due to the metabolite of Bu (rather than Bu itself) either directly or indirectly through depletion of the cellular GSH pool. In fact, it has been suggested by DeLeve et al[17] that hepatic toxicity of Bu could be due to either GSH depletion or the GSH-conjugated metabolite of Bu, depending on the GSH status of the cell. We did not find any difference in the pre-BMT hepatic GSH levels in the liver in 58 patients with and without HVOD for whom the data were available. It may be more appropriate to assess postconditioning SEC/hepatic GSH levels and their relationship with hepatic toxicity possibly in other models. It is of interest to note that SECs are more vulnerable to GSH-depleting drugs than hepatocytes, the latter being more efficient in synthesizing GSH than SECs.[28]

For Bu Cl/F to be higher, elevated levels of GSTA1 would be expected, as this is the major isoenzyme involved in the metabolism of Bu.[13,14] We have shown a 2- to 4-fold increase in the enzyme and mRNA levels of GSTA1 in patients with β-thalassemia major with the GSTM1-null genotype compared with those with the GSTM1-positive genotype.[29] This would result in enhanced metabolism of Bu and GSH depletion, thereby causing increased toxicity in these patients. It has also been reported by Ritter et al[30] that elevated expression levels of GSTA1-1 in patients treated with

From www.bloodjournal.org by on January 28, 2011. For personal use only.

Bu could accelerate tissue factor expression due to injury to the SECs, shifting these patients to a procoagulative state. They postulated that variability in the expression of GST-α could explain the unpredictable occurrence of HVOD. Nevertheless, we cannot exclude the possibility that the GST-μ enzyme encoded by the GSTM1 locus could play a major role in hepatocytes/SEC protection, and its absence in individuals with the null genotype could be detrimental to these cells.

McDonald et al[10] have recently shown an association between the AUC of Cy and its metabolites and the incidence of hepatic toxicity. Our data clearly suggests that Bu or its metabolites cause hepatic toxicity that may be worsened by subsequent exposure to Cy, resulting in HVOD. It would be important to evaluate the impact of Bu and Cy individually and sequentially on hepatic expression of GST enzymes in animal models to define the role and interaction of these drugs in causing hepatic/SEC toxicity.

The overall frequencies of the GSTM1- and GSTT1-null genotypes in these patients were not significantly different from those seen in the healthy controls (37.7% vs 33% had the GSTM1-null genotype, 29% vs 26% had the GSTT1-null genotype, and 9.6% and 8% had both null genotypes among 114 thalassemic patients and 250 healthy controls, respectively), quite similar to those reported for Asian Indian population,[31,32] and the frequency of the GSTM1-null genotype is lower than that has been reported for another Caucasian population (33% vs 50%; $P = .003$).[16] Hence, the high incidence of the GSTM1-null genotype in thalassemic patients with HVOD cannot be attributed to increased frequency of this genotype in the Indian population.

We conclude that the GSTM1-null genotype is an independent risk factor for HVOD in patients with β-thalassemia major undergoing BMT. Its identification prior to BMT could allow individualization of therapy with regard to conditioning regimen and prophylaxis for HVOD.

Acknowledgment

We would like to acknowledge the help provided by Ms M. L. Kavitha in statistical analysis.

References

1. Wadleigh M, Ho V, Momtaz P, Rishardson P. Hepatic veno-occlusive disease- pathogenesis, diagnosis and treatment. Curr Opin Hematol. 2003; 10:451-462.
2. DeLeve LD, Shulman HM, McDonald GB. Toxic injury to the hepatic sinusoids: sinusoidal obstruction syndrome (veno-occlusive disease). Semin Liver Dis. 2002;22:27-42.
3. Bearman SI. Avoiding hepatic veno-occlusive disease: what do we know and where are we going? Bone Marrow Transplant. 2001;27:1113-1120.
4. Barker CC, Butzner JD, Anderson RA, Brant R, Sauve RS. Incidence, survival and risk factors for the development of veno-occlusive disease in pediatric hematopoietic stem cell transplant recipients. Bone Marrow Transplant. 2003;32:79-87.
5. Carreras E. Veno-occlusive disease of the liver after hemopoietic cell transplantation. Eur J Haematol. 2000;64:281-291.
6. Santos GW, Tutschka PJ, Brookmeyer R, et al. Marrow transplantation for acute non-lymphocytic leukaemia after treatment with busulfan and cyclophosphamide. N Engl J Med. 1983;309:1347-1353.
7. Clift RA, Buckner CD, Thomas ED, et al. Marrow transplantation for chronic myeloid leukemia: a randomized study comparing cyclophosphamide and total body irradiation with busulfan and cyclophosphamide. Blood. 1994;84:2036-2043.
8. DeLeve LD. Hepatic venoocclusive disease: a major complication of hematopoietic stem cell transplantation in cancer patients. Tumori. 2001; 87:S28-S30.
9. Slattery JT, Sanders JE, Buckner CD, et al. Graft rejection and toxicity in relation to busulfan pharmacokinetics. Bone Marrow Transplant. 1995;16: 31-42.
10. McDonald GB, Slattery JT, Bouvier ME, et al. Cyclophosphamide metabolism, liver toxicity and mortality following hematopoietic stem cell transplantation. Blood. 2003;101:2043-2048.
11. Hassan M, Ljungman P, Ringden O, et al. Effect of busulfan on the pharmacokinetics of cyclophosphamide and its 4-hydroxy metabolite: time interval influence on therapeutic efficacy and therapy related toxicity. Bone Marrow Transplant. 2000;25:915-924.
12. Dirven HA, Van Ommen B, Van Bladeren PJ, et al. Glutathione conjugation of alkylating cytostatic drugs with a nitrogen mustard group and the role of glutathione S-transferases. Chem Res Toxicol. 1996;9:351-360.
13. Czerwinski M, Gibbs JP, Slattery JT. Busulfan conjugation by glutathione S-transferases α, μ, and π. Drug Metabol Disp. 1996;24:1015-1019.
14. Gibbs JP, Czerwinski M, Slattery JT. Busulfan-glutathione conjugation catalyzed by human liver cytosolic glutathione S-transferases. Cancer Res. 1996;56:3678-3681.
15. Nelson HH, Wiencke JK, Christiani DC, et al. Ethnic difference in the prevalence of the homozygous deleted genotype of GST. Carcinogenesis. 1995;16:1243-1245.
16. Arruda VR, Grignolli CE, Gonclaves MS, et al. Prevalence of homozygosity for the deleted alleles of GSTM1 and GSTT1 among distinct ethnic groups from Brazil: relevance to environmental carcinogenesis? Clin Genet. 1998;54:210-214.
17. DeLeve D, Wang X. Role of oxidative stress and glutathione in busulfan toxicity in cultured murine hepatocytes. Pharmacology. 2000;60:143-154.
18. Poonkuzhali B, Srivastava A, Quernin MH, et al. Pharmacokinetics of oral busulphan in children with beta thalassemia major undergoing allogeneic bone marrow transplantation. Bone Marrow Transplant. 1999;24:5-11.
19. Li CK, Yuen PM, Wong R, et al. Busulphan level and early mortality in thalassaemia patients after BMT. Bone Marrow Transplant. 1999;23:307-310.
20. Jones RJ, Lee KSK, Beschorner WE, et al. Veno-occlusive disease of the liver following bone marrow transplantation. Transplantation. 1987;44: 778-783.
21. Lucarelli G, Galimberti M, Polchi P, et al. Bone marrow transplantation in patients with thalassemia. New Engl J Med. 1990;332:417-421.
22. Quernin MH, Poonkuzhali B, Medard Y, et al. High-performance liquid chromatographic method for quantification of busulfan in plasma after derivatization by tetrafluorothiophenol. J Chromatogr. 1999;721:147-152.
23. Heinzel G, Woloszezak R, Thoman P. Pharmacokinetic pharmacodynamic data analysis system for the PC. Stuttgart, Germany: Gustav Fischer; 1993.
24. Pfeiffer CM, Huff DL, Gunter EW. Rapid and accurate HPLC assay for total plasma homocysteine and cysteine in a clinical laboratory setting. Clin Chem. 1999;45:290-292.
25. Arand M, Mullbauer R, Hengstler J, et al. A multiplex polymerase chain reaction protocol for the simultaneous analysis of the GSTM1 and GSTT1 polymorphisms. Anal Biochem. 1996;236:184-186.
26. Dean AG, Dean JA, Coulombier D, et al. Epi Info version 6: a word processing database and statistical program for epidemiology on microcomputers. Atlanta, GA: Centers for Disease Control and Prevention; 1994.
27. Slattery JT, Kalhorn TF, McDonald GB, et al. Conditioning regimen-dependent disposition of cyclophosphamide and hydroxycyclophosphamide in human marrow transplantation patients. J Clin Oncol. 1996;14:1484-1494.
28. DeLeve LD, Wang X, Kuhlenkamp JF, Kaplowitz N. Toxicity of azathioprine and monocrotaline in murine sinusoidal endothelial cells and hepatocytes: the role of glutathione and relevance to hepatic veno-occlusive disease. Hepatology. 1996;23:589-599.
29. Poonkuzhali B, Lapoumeroulie C, Shaji RV, et al. GSTM1 null genotype is associated with elevated GST α expression in children with beta thalassemia major [abstract]. Blood. 2002;100:4373b. Abstract 4373.
30. Ritter CA, Sperker B, Grube M, et al. Overexpression of glutathione S-transferase A1–1 in ECV 304 cells protects against busulfan mediated G2-arrest and induces tissue factor expression. Br J Pharmacol. 2002;137:1100-1106.
31. Sreelekha TT, Ramadas K, Pandey M, Thomas G, Nalinakumari KR, Pillai MR. Genetic polymorphism of CYP1A1, GSTM1 and GSTT1 genes in Indian oral cancer. Oral Oncol. 2001;37:593-598.
32. Garte S, Gaspari L, Alexandrie A-K, et al. Metabolic gene polymorphisms in control populations. Cancer Epidemiol Biomarkers Prev. 2001;10: 1239-1248.