Selected Summary

Glutathione S-Transferase M1 polymorphism – A risk factor for hepatic venoocclusive disease in bone marrow transplantation.

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SUMMARY

This prospective study was designed to evaluate polymorphisms of the enzyme glutathione- S-transferase as a risk factor for the development of hepatic veno occulsive disease (HVOD) in beta thalessemia patients undergoing allogeneic bone marrow transplantation. It was conducted by the department of haematology, Christian Medical College Vellore, India between the years 1995 and 2002. 114 consecutive patients of β-thalessemia undergoing allogeneic bone marrow transplantation were included in the study. The conditioning regimen consisted of busulfan 16mg/kg or 600mg/m^2 in four divided doses on days –9 to –6 and cyclophosphamide 50mg/kg on days –5 to –2. Anti thymocyte globulin (ATG) 30mg/kg was given on days–4 to –2 only to those receiving busulfan at 16mg/kg. Hepatic veno-occlusive disease was diagnosed based on the Batimore criteria.[1].

Glutathione S-transferase, the hepatic enzyme responsible for the metabolism of both busulfan and cyclophosphamide, consists of four sub families – GST A1, M1, P1 and T1.[5,6] Of these, GST MI and TI are highly polymorphic with homozyous deletion of either or both genes occuring at varying but significant frequencies in different ethnic groups.[7] Genomic DNA from peripheral blood of all patients was collected pre-BMT from all patients and 250 healthy controls for simultaneous amplification of GSTMI and GSTTI genes by multiplex PCR. In order to assess the impact of the polymorphisms on busulfan pharmacokinetics, busulfan levels were measured by HPLC (high pressure liquid chromatography). All patients were subjected to a pre-transplant liver biopsy and hepatic glutathione levels were measured by HPLC. Statistical significance of difference between groups was calculated by Chi square, Fischer's exact or students' t-test as applicable. Multivariate logistic regression analysis was done for the possible predictors of HVOD as independent variables.

33 patients had hepatic veno occlusive disease. The incidence of HVOD was higher in those who had GST MI null genotype compared to those with GSTMI positive genotype (46.5% vs 18.3% p=0.001). There was no significant difference in incidence of HVOD in GST TI null or positive genotypes (18% vs 33.5% P=0.13). GST MI null genotype was seen in 6 of the 8 patients who had severe HVOD (75%).

The clearance (CL/F) of oral busulfan after 1st dose was higher in those with HVOD than those without HVOD. (0.403±0.06 vs 0.33±0.71 L/h/kg p=0.00001). The steady state concentrate (Css) of busulfan was lower in those with HVOD than those without (508±125 vs 656±255 ng/ml p=0.001). Those with a GSTMI null phenotype had a higher clearance (0.40±0.064 vs 0.33±0.071 p=0.00001) and a lower steady state concentration (544±184 vs 667±256 p=0.001). Pre-transplant hepatic glutathione levels in liver biopsy specimens showed no significant difference between those with or without HVOD GST MI null genotype and age were the only two significant risk factors for HVOD on multivariate analysis.

The frequencies of GST MI and GST TI null genotypes were similar compared to that of the healthy controls and also to that reported for Asian population.

COMMENTS

Hepatic veno occlusive disease (HVOD) is an important complication of bone marrow transplantation. The reported incidence of this complication varies between 10 and 20%.[1-3] and in thalessemic patients with pre-existing hepatic dysfunction can be as high as 45%. The pathogenesis of HVOD is not fully understood but it is considered to occur due to the damage to the sinusoidal endothelial cells (SEC) and the surrounding centrilobar hepatocytes, usually mediated by the conditioning regimen. The enzyme glutathione S-transferase is responsible for the hepatic metabolism of both busulfan and cyclophosphamide.

Busulfan is metabolized by GST to form positively charged sulfonium ion which is cleaved to a lipophilic compound tetrahydrothiophene which is toxic to SEC and hepatocytes. As busulfan is given before cyclophosphamide, the metabolism of busulfan is responsible for depletion of hepatic cytosolic glutathione which is necessary for detoxification of cyclophosphamide. This can lead to cyclophosphamide mediated hepatic injury.[4]

This study establishes GST MI null genotype as a definite risk factor for HVOD. There are 2 possible explanations for this. One is that, absence of GST MI isoform increases the level of GSTAI isoform which is the most active isoform of the enzyme.[5,6] This can lead to increased production of the toxic busulfan metabolite and greater depletion of cytosolic glutathione. The other hypothesis is that GSTMI genotype codes for an enzyme that could be protective to the SEC / hepatocytes. The clearance of busulfan is higher and the steady state concentration lower in those with GST MI null genotype establishing the fact that busulfan metabolism is accelerated in this genotype. The pre BMT hepatic glutathione-level did not show any correlation with HVOD as glutathione depletion occurs only after exposure to the drugs. Hence a hepatic glutathione level post conditioning in all patients and its correlation with HVOD would have been ideal.

The importance of this study lies in its potential for therapeutic application. Prospective bone marrow transplant patients who are positive for GST MI null genotype should have their conditioning regimen modified in order to prevent this serious and sometimes fatal complication.

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