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Effect of glutathione *S*-transferases on the survival of patients with acute myeloid leukaemia

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Abstract

The objective of the study was to investigate the effect of genetic polymorphisms in glutathione *S*-transferases (GST) on the survival of acute myeloid leukaemia patients receiving adriamycin induction therapy. A total of 89 patients were included in the study. Patients who carried at least one GSTM1 allele had trend towards a better survival (mortality rate ratio (RR) 0.588; 95% CI 0.334–1.036) than GSTM1 *0/0 patients. However, at low accumulated adriamycin dose, GSTM1 *0/0 cases had a better survival than people expressing the gene (RR = 6.1; 95% CI = 1.2–11.0). The GSTT1 and GSTP1 genotype did not influence the survival in any of the groups.

Keywords: Leukaemia; Chemotherapy; Survival; GST (glutathione *S*-transferase)

1. Introduction

Glutathione *S*-transferases (GST) are involved in the detoxification of xenobiotic compounds by enzymatic conjugation of the compounds or their metabolites with reduced glutathione, and confer protection against oxidative stress as these enzymes are efficient in catalysing the major toxic metabolites formed by lipid peroxidation (Hayes and McLellan, 1999). GST are also involved in the metabolism of many cytotoxic cancer therapeutic agents, and it has been suggested that the difference in response to chemotherapy was in part due to difference in the level and distribution of GST enzymes.

Seven different GST gene families have been described, and genetic polymorphisms have been detected in several of these families (Strange et al., 2000). Homozygous deletion of the GSTM1 and GSTT1 genes has been observed in 50% and 10%, respectively, in the Danish population. A single nucleotide polymorphism within the coding region (codon 105) has been reported in the GSTP1 enzyme, leading to an amino acid change from Ile to Val (Board et al.,1989), resulting in a changed substrate affinity. The frequency of the heterocygotes for Ile and homo- and heterocygotes for Val in the Danish population was 42% and 58%, respectively.

A few epidemiological studies have investigated the role of genetic variants of GST as risk factors in the development of leukaemia, but the results are not clear-cut and may depend on the histological diagnosis. In a study of adult acute leukaemia, the GSTM1 and GSTT1 deletions were associated with an increased risk (Rollinson et al., 2000), whereas deletion of GSTM1 and GSTT1 was not linked to an increased risk of acute myeloid leukaemia (AML) (Crump et al., 2000). However, the deletion of GSTT1 was linked to a fourfold increased risk for developing myelodysplasia, a clonal proliferative disorder that often progresses to AML (Chen et al., 1996). In the Danish AML cohort, the GSTM1, GSTT1 and GSTP1 genotypes were not identified as risk factors (Autrup, unpublished results). It has been hypothesized that GST enzymes are disease-modifying rather than disease-causing genes (Hayes and Strange, 2000).

GST are involved in the metabolism of several types of anticancer drugs, and increased GST activity has been implicated as a drug resistance mechanism in cell lines through increased detoxification of, e.g. alkylating agents. There are several examples that the regulation of the GST enzymes is altered in tumour tissues compared to normal tissues, e.g. the

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level of GSTP1 is increased in many tumours, e.g. colorectal tumours.

It has also been shown that the GST levels have a prognostic value, e.g. GSTA and GSTM is down regulated in colorectal tumours. In this study, we have investigated the role of the genetic variants of GST on the survival of AML patients receiving anthracyclin as induction therapy.

2. Material and methods

Patients attending the Department of Hematology and diagnosed with acute myeloid leukaemia in the period April 1980 to May 1989 were included in the study. The study group was restricted to patients receiving cytoreductive drugs, i.e. anthracyclin and cytarabin (Ara–C), as induction therapy and who survived for more than one month after diagnosis. The group consisted of 40 males and 49 females. The FAB classification was used to describe the disease status at the patients' entry into the study. Cytogenetic characterisation of the patient material was incomplete. The mean age at diagnosis was 58 years and the range 24–80. The median cumulative dose of anthracyclin was 370 mg, and patients receiving less than the median dose were classified as low dose and greater than 370 mg as high dose.

Bone marrow cells were obtained at the time of diagnosis and were stored in liquid nitrogen until analysis.

The project was approved by the ethical committee in the County of Århus.

DNA was isolated from bone marrow cells by a phenol extraction procedure (Spurr et al., 1991). The genotypes of GSTM1, GSTT1 and GSTP1 (Ile105Val) were determined as previously described by Autrup et al. (1999).

The effect of the metabolic genotype on the survival was analysed by the Cox-regression analysis. The survival curve was adjusted for the age of patients at entry. The interaction between drug dose and genotype on survival was analysed by chi-square analysis.

3. Results

The effect of the GSTM1, GSTT1 and GSTP1 on the survival of AML patients treated with anthracyclins and Ara–C was analysed by survival analysis. In this case group, 50 patients (56%) were classified as GSTM1 *0/0 and 39 (44%) as GSTM1 *1, 15 patients (17%) were GSTT1 *0/0 and 74 were GSTT1 *1/. In the case of GSTP1, 35 patients (39%) were classified as GSP1 Ile/Ile and 54 (61%) as Ile/Val or Val/Val. The distribution of the GSTM1 genotypes was not statistically significantly different in FAB classification groups M1, M2, M6 combined and M4, M5 combined. During the first year of diagnosis, there was no difference in survival between the GSTM1 *0/0 (50 cases) and GST M1 *1 (39 cases) groups (Fig. 1a), but overall a trend towards a significantly better survival was observed in the

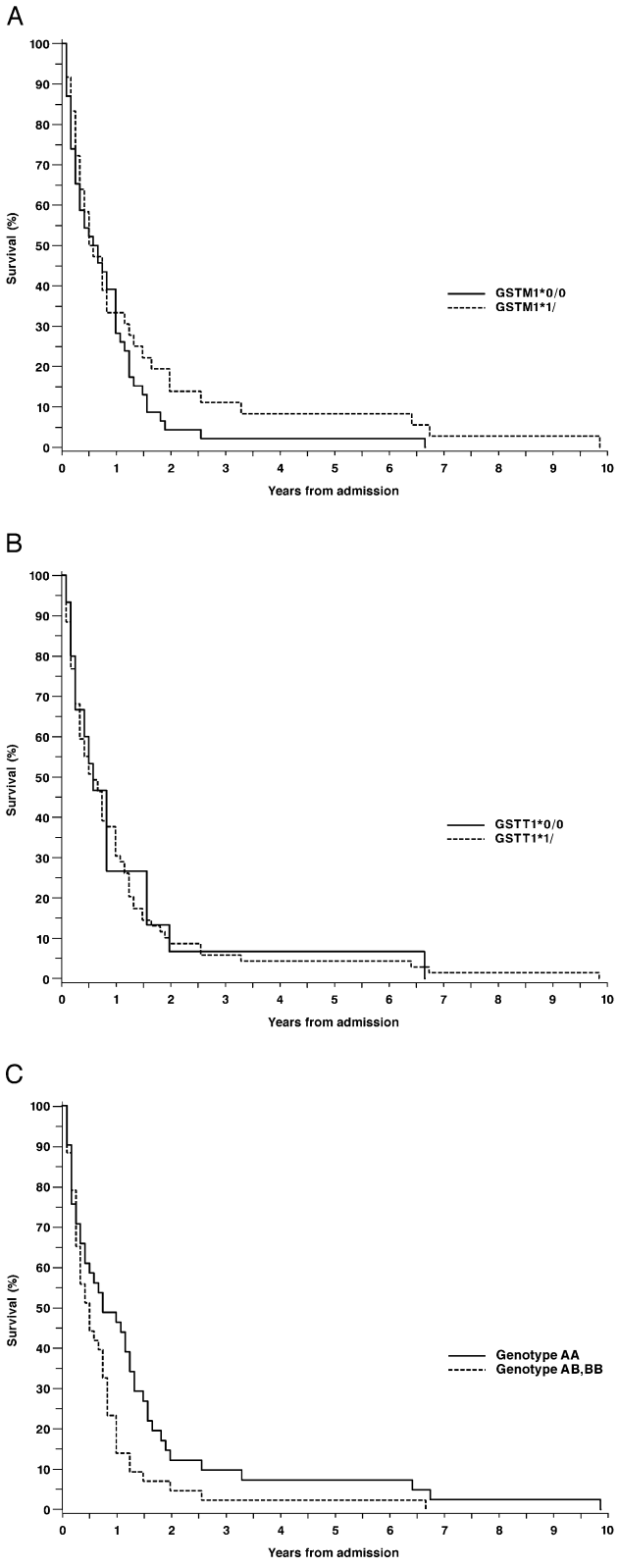

Fig. 1. The effect of GST genotypes on the survival of acute myeloid leukaemia patients treated with anthracyclines as induction therapy. (A) GSTM1, (B) GSTT1, (C) GSTP1.

GSTM1 *1 group (risk ratio 0.588; 95% CI 0.334–1.036), whereas carriers of at least one GSTT1 allele did not have an altered survival (Fig. 1b) (RR = 1.143; 95% CI 0.595–2.193). A slightly poorer survival was observed in patients having at least one low activity GSTP1 allele (Fig. 1c) (RR = 1.554; 95% CI 0.932–2.591). Due to the low frequency of the GSTT1 *0/0 in the population and the number of patients enrolled in the study, the interaction of GSTT1 and GSTM1 genotypes could not be investigated. The survival curves were adjusted for age.

The interaction of GST genotypes and the accumulated dose of the drug on survival have been investigated. The cases were stratified according to survival either as less than 12 months or longer than 12 months after diagnosis. All patients received the induction therapy consisting either of antracyclin alone or in combination with Ara–C. Only patients with relapse received a more complex treatment. A statistically significant increased survival was observed in the high-dose group compared to the low-dose group when all genotypes were combined. However, in the low-dose group, GSTM1 *0/0 patients (16 cases) had a significantly better survival compared to the patients expressing at least one copy of the gene (RR = 6.1; 95% CI 1.2–11.0), whereas GSTT1 *0/0 patients (3 cases) did not have a better survival (RR = 1.37). Furthermore, carrier of at least one GSTP1 Val allele (16 cases) had a better survival at the low level than patients having the Ile105Ile genotype (RR = 7.5; 95% CI 1.61–24.53).

4. Discussion

Adriamycin, used in the induction therapy of acute myeloid leukaemia patients, is an antracyclin antineoplastic agent. It exerts its cytotoxic effects through direct interaction with DNA causing DNA strandbreaks and by accelerating free radical mediated lipid peroxidation (DeAtley et al., 1998; Papageorgiou et al., 1998). Anthracyclines are quinone-containing compounds that undergo redox cycling leading to the formation of reactive oxygen species and subsequent lipid peroxidation (Müller et al., 1997). The GST enzymes may contribute to the resistance by catalysing the detoxification of toxic lipid peroxidation products. In children with acute lymphoblastic leukaemia, it was observed that patients expressing GSTM1 activity in lymphoblasts had a threefold increased risk of relapse compared to patients not expressing the enzyme (Hall et al., 1994). These patients were treated by the standard Medical Research Council (MRC) protocols, e.g. 6-mercaptopurine, methotrexate. In contrast to this observation, we found that the GSTM1 *0/0 genotype was associated with a poorer survival. A similar observation was observed in the case of epithelial ovarian cancer, where the combination of GSTM1 null and GSTT null was associated with a poorer survival. It was suggested that it might be a result of unresponsiveness to primary chemotherapy in the group of patients with the null/null genotype (Howells et al., 1998). In the case of childhood AML, patients classified as GSTT1 *0/0 had a reduced survival compared to patients with at least one GSTT1 allele (Davies et al., 2001).

It has been proposed that the low level of the GSTP1 enzyme was a prognostic factor for response to chemotherapy and prolonged survival in the case of colorectal cancer (Mulder et al., 1995), and positive staining for GSTP was a significant risk factor for chemotherapy resistance in the case of non-small cell lung cancer (Nakanishi et al., 1999). In our study, the focus was on a specific allelic variant and not total enzyme activity. Furthermore, low content of GSTP in chronic B-cell lymphoproliferative disorder could explain the sensitivity of this disease to alkylating agents (Marie et al., 1995). There are several examples both in vivo and in vitro, that GSTP1 expression is linked to resistance against chemotherapy, i.e. transfection of antisense GSTP1 cDNA decreased the expression of GSTP1, and the cells showed a fourfold increased sensitivity to adriamycin, whereas the sensitivity of 5-fluorouracil was not altered (Ban et al. 1996; Niitsu et al., 1998)). The mechanism of GSP1 protection against oxidative stress-induced cell death is mediated by the regulation of stress kinases (Yin et al., 2000). In our study, a slightly better survival was observed in GSTP1 Ile/Ile patients compared with patients with the Ile/Val or Val/Val genotypes. Whereas previous studies have focused on the overall enzyme activity, we do not know the level of expression of the enzyme. However, the different GSP1 genotypes have different substrate specificity, so even at the same level of expression, the different ability to detoxify the mediator of cytotoxicity is anticipated depending on the substrate. However, the role of the allelic variants in the detoxification of oxidant species that may mediate the cytotoxic effect is unknown. A longer survival in patients treated with a low dose of the drug and having at least one GSTP1 105Val allele suggests a role of this enzyme in the detoxification and thus influence the pharmacological level of the active species. In contrast, a study of breast cancer patients receiving either chemotherapy or radiation patients with the low activity Val/Va1 genotype had a better survival (Sweeney et al., 2000). The chemotherapy used in the breast cancer study was not only adriamycin but also alkylating agents and 5-fluorouracil.

Recently, the response of patients with childhood acute lymphoblastic leukaemia to glucocorticoid treatment has been studied in relationship to GST genotype. Patients with the GSTT1 null genotype had a sixfold reduced risk of relapse whereas the GSTP1 and GSTM1 genotype did not have any effect (Anderer et al., 2000). It was suggested that the effect was due to a direct interaction of GSTT1 with the glucucorticoids. In our study, GSTT1 genotype did not have any effect on the survival of patients treated with anthracyclins; however, patients with the GSTT1 * 0/0 genotype and receiving low dose of the drug had a slight, but significant increased survival in contrast to patients receiving high dose. In contrast to the effect of GST enzymes on the survival after treatment with anthracyclins, especially in low doses, genetic polymorphism in *N*-acetyltransferase 2

(NAT2) and cytochrome P4502D6 (CYP2D6) enzymes did not have any effect on the survival of the AML patients (Autrup et al., unpublished results). Although genetic polymorphism in these genes is known for influencing the pharmacological active dose of drugs metabolised by these enzymes, they do not appear to play a role in the metabolism of anthracyclins.

Treatment of cells in vitro with adiamycin results in the induction of, e.g. glutathione *S*-transferases, and this induction may in part be related to the resistance as the induced enzymes are involved in the protection of the cytotoxic effect that is the desired pharmacological response (Papageorgiou et al., 1998). GSTM1 does not appear to be induced in xenografts of childhood hepatoblastoma by treatment with adriamycin (Bader et al., 1998). It may also be feasible that the GST isozymes themselves do not offer the selective advantages, but one or more of the GST genes are linked to the expression of other genes coding for proteins that are involved in the clinical course of the disease.

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