SHORT COMMUNICATION

Induction of sister chromatid exchange by 1,2-epoxy-3-butene in cultured human lymphocytes: influence of GSTT1 genotype

Sabrina Bernardini, Ari Hirvonen, Katarina Pelin¹ and Hannu Norppa²

Department of Industrial Hygiene and Toxicology, Finnish Institute of Occupational Health, Topeliusenkatu 41 a A, FIN-00250 Helsinki, Finland

¹Present address: Department of Medical Genetics, University of Helsinki and the Folkhålsan Institute of Occupational Health, Topeliuksenkatu 41 a A, FIN-00250 Helsinki, Finland

²To whom correspondence should be addressed

Email: hannu.norppa@occuphealth.fi

The influence of glutathione S-transferase T1 (GSTT1) genotype on the genotoxicity of 1,2-epoxy-3-butene (MEB), a metabolite of 1,3-butadiene, was assessed by the analysis of sister chromatid exchanges (SCEs) in 72-h human whole-blood lymphocyte cultures. The cultures were from 18 donors, representing both GSTT1 ‘positive’ genotype (with at least one undeleted GSTT1 allele; GSTT1 activity present) and GSTT1 ‘null’ genotype (homozygous deletion of the GSTT1 gene; no GSTT1 activity). As we have previously observed that alleleism of glutathione S-transferase M1 (GSTM1) affects SCE induction by MEB in cultured lymphocytes, only individuals with the GSTM1 null genotype were included in this study. At 125 and 250 µM MEB (treatment at 24 h for 48 h), the mean frequencies of MEB-induced SCEs per cell (control level subtracted) were 4.5 (SD 1.8) and 8.9 (SD 1.0) for GSTT1 null cell cultures (n = 5) respectively, and the difference between the genotypes was statistically significant (P < 0.001) at the higher dose. All individual mean frequencies of SCEs induced by 250 µM MEB were higher in the GSTT1 null group (range 11.2–13.9) than in the GSTT1 positive group (range 7.2–10.8). The findings suggest that GSTT1, in addition to GSTM1, is involved in the detoxification of MEB in human whole-blood lymphocyte cultures. The deletion of the GSTT1 gene results in reduced erythrocytic detoxification capacity, thereby increasing the genotoxic effects of MEB.

Occupational exposure to 1,3-butadiene, a petrochemical widely used in the production of rubber and plastics, has been implicated to play a role in the development of human cancer (1–3). Butadiene is metabolized via cytochrome P450 mixed-function oxidases to 1,2-epoxy-3-butene (MEB*), which is further converted into 1,2,3,4-diepoxybutane (DEB) or, via epoxide hydrolase, to 3-butene-1,2-diol (1). While both MEB and DEB are genotoxic in a variety of mammalian and bacterial assays, DEB has in most studies been shown to be a much more potent genotoxin than MEB (1,4–7).

Individual risk for genotoxic and carcinogenic effects is expected to depend on environmental and genetic factors that modify the balance between metabolic activation and detoxification (8). Glutathione S-transferases (GSTs) are a family of enzymes that show genetic polymorphisms in humans and have been suggested to be involved in the detoxification of butadiene metabolites. Specific class µ and θ isozymes, GSTM1 and GSTT1, are lacking in ~50% and 10–20% of Caucasians, respectively, due to homozygous deletions (null genotype) of their genes (9–11). The GSTM1 and GSTT1 null genotypes have been associated with an increased risk of various cancers (8,12–16).

Our previous studies have shown a higher induction of sister chromatid exchanges (SCEs) by MEB in lymphocyte cultures from GSTM1 null donors in comparison with GSTM1 positive donors (having at least one undeleted GSTM1 allele), suggesting that GSTM1 is involved in the detoxification of MEB (17). GSTT1 polymorphism has been shown to be the main reason for individual differences in the in vitro genotoxic effects of DEB (18–23). Neither GSTT1 nor GSTM1 genotypes influenced SCE induction by 3,4-epoxybutane-1,2-diol, another epoxide metabolite of BD (24).

The aim of the present study was to characterize the possible role of the GSTT1 genotype in determining individual SCE response to MEB in cultured human lymphocytes. Although it is not known if MEB is a substrate for GSTT1, DEB-sensitive subjects have also been described to show a slightly increased sensitivity to SCE induction by MEB (5). Since the GSTT1 null genotype is a major determinant of DEB sensitivity (18–23), the observed increased genotoxic response to MEB may similarly be explained by alleleism of GSTT1.

For this purpose, 6-ml whole-blood lymphocyte cultures, duplicate for each treatment and donor, were established (25) in air-tight culture bottles from heparinized blood samples of 18 GSTM1 null volunteers representing both GSTT1 positive (n = 13) and GSTT1 null (n = 5) genotypes. The genotypes of the donors had earlier been determined by multiplex polymerase chain reaction (19,28). Two final concentrations of MEB were chosen for the experiment on the basis of a previous investigation (17) and a dose-range study. The cultures were treated 24 h after culture initiation by either 125 or 250 µM MEB (98%; Aldrich, Milwaukee, WI; first dissolved in phosphate-buffered saline; treatment solutions prepared in serum-free growth medium and administered at a volume of 0.5 ml) or 0.5 ml of serum-free growth medium and were further incubated at 37°C for a total culture time of 72 h. The cells were harvested, fixed, put on microscopic slides, and stained by a modification of the fluorescence-plus-Giemsa technique, as described earlier (24,25).

For each culture, one microscopist scored the frequency of SCEs from 25 second division metaphases (50 cells/donor and treatment) and replication index (RI; mean number of replications completed by the scored metaphases) from 100 cells/culture (200 cells/donor and treatment), using coded slides. Two-way analysis of variance (ANOVA) was used for

© Oxford University Press

testing the effect of the GSTT1 genotype and treatment on SCEs and RIs.

The results of the SCE analysis of MEB-treated lymphocyte cultures of the 18 donors are presented in Table I. A highly significant (P < 0.001) increase in SCEs was obtained by both concentrations of MEB in all cultures except one GSTT1 positive donor at 125 µM MEB (Table I).

At 250 µM MEB, a higher mean frequency of SCEs/cell was observed in the cultures from GSTT1 null donors as compared with those from the GSTT1 positive donors (Table I). When SCEs induced by MEB were calculated by subtracting each donor’s control SCE level (Figure 1), the GSTT1 null cells showed, at 250 µM MEB, a 1.4-fold higher mean number of induced SCEs/cell than those with the GSTT1 positive genotype (12.5 versus 8.9; P < 0.001); all individual SCE values induced by 250 µM MEB were higher in the GSTT1 null (range 11.2–13.9) than in the GSTT1 positive (7.2–10.8) genotype group. No clear difference between the genotypes was detected at 125 µM MEB.

RIs decreased slightly in all cultures treated with MEB (treatment effect significant at P < 0.001), but the GSTT1 genotype had no influence. In the control cultures, no genotype effects could be seen for SCEs or RIs (Table I).

The present results indicate a role for GSTT1 in the detoxification of MEB, which is in agreement with previous findings of Wiencke and Kelsey (5) on an increased SCE response to MEB in lymphocyte cultures of DEB-sensitive subjects. The magnitude of the genotype effect in MEB-induced SCEs effect was roughly similar in the two studies (1.4 versus 1.3–2.0-fold at different concentrations), although Wiencke and Kelsey (5) could use clearly higher concentrations (500–2000 µM) than we could, possibly due to different experimental design.

As GSTT1 polymorphism could have confounded our previous study on MEB and GSTM1 genotype (17), we decided to determine the GSTT1 genotypes of the donors examined in the earlier paper. Two of the six GSTM1 null donors were also found to be GSTT1 null, while no GSTT1 nulls were present.
among the six GSTM1 positive individuals. Since the four GSTM1 null/GSTT1 positive donors still showed a 1.4 times higher mean number of induced SCEs/cell than the GSTM1 positive donors (31.0 versus 22.3; at 250 μM MEB), the previously observed association between MEB-induced SCEs and GSTM1 polymorphism is unlikely to be explained by the GSTT1 polymorphism. Thus, GSTM1 and GSTT1 appear to be equally involved in the detoxification of MEB in human whole-blood lymphocyte cultures.

A very clear effect of the GSTT1 (but not GSTM1) genotype was previously described for the induction of SCEs and micronuclei in human lymphocyte cultures by DEB (18–23). As GSTT1 activity is mostly found in erythrocytes, no influence of the GSTT1 genotype on DEB-induced SCEs could be shown in cultures of isolated human lymphocytes (22). Given the high activity of GSTT1 in erythrocytes, individual GSTT1 genotype could particularly modulate the genotoxic effects of its substrates in blood-forming organs and peripheral blood. Actually, an increase in chromosome aberrations was observed in peripheral lymphocytes of GSTT1 null (but not in GSTT1 positive) workers exposed to butadiene, indicating that GSTT1 deficiency may play a role also in vivo (26). As GSTT1 seems to be involved in the detoxification of both DEB and MEB, the chromosome damage seen in butadiene-exposed GSTT1 null individuals could reflect the effect of one or both of these metabolites. On the other hand, no interaction could be demonstrated between occupational butadiene exposure and lymphocyte SCEs (26,27) and for micronuclei a lower frequency was observed in GSTT1 null than in GSTT1 positive butadiene-exposed workers (26).

In this connection, it is interesting to note that the GSTT1 null genotype is highly over-represented in myelodysplastic syndromes: clonal pre-cancerous proliferative disorders that may be induced by occupational and environmental exposures (28). The possible carcinogenicity of butadiene in humans has also been associated with an increase of lymphohematopoietic cancers (1–3).

In addition to glutathione conjugation, hydrolysis by epoxide hydrolase may provide another detoxification route for butadiene epoxides. The human microsomal epoxide hydrolase is polymorphic, and expression studies in transfected bacteria (29) have suggested that allelic variants of the human microsomal epoxide hydrolase gene (EPHX) correlate with different enzyme activities. Further studies are required to judge if the EPHX genotype also affects individual ability to metabolize butadiene epoxides in human cells.

In conclusion, the present study showed that the genotoxicity of MEB (a metabolite of butadiene) in human lymphocyte cultures depends on the GSTT1 genotype of the donor. As a similar result has previously been obtained for the GSTM1 genotype, it appears that both GSTT1 and GSTM1 are involved in the detoxification of MEB in cultured human lymphocytes. The null genotypes of the two GSTs were associated with a 1.4-fold increase in SCEs at a high in vitro concentration of 250 μM MEB. Although one study has indicated an effect of the GSTT1 polymorphisms on chromosome damage also in butadiene production workers, it remains unclear if GST polymorphisms modulate the genotoxic or carcinogenic effects of butadiene in humans in vivo.

Acknowledgements

We wish to thank Ms Hilka Järventaus for the blood sampling and the blood donors for participating in the study. The visit of S.B. to Finland was supported by the Centre for International Mobility (CIMO) under the European Scholarship Programme of the Ministry of Foreign Affairs.

References

S. Bernardini et al.


Received on July 3, 1997; revised on October 9, 1997; accepted on October 9, 1997