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SHORT COMMUNICATION

# Influence of *GSTM1* genotype on sister chromatid exchange induction by styrene-7,8-oxide and 1,2-epoxy-3-butene in cultured human lymphocytes

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**Glutathione S-transferase M1 (GSTM1), catalyzing the conjugation of various reactive molecules with glutathione (GSH), shows genetic polymorphism in humans. Almost half of all Caucasians lack the *GSTM1* gene, being theoretically at a higher risk from the toxic effects of substrates for GSTM1. The purpose of the present study was to investigate whether the *GSTM1* genotype of lymphocyte donors influences the *in vitro* induction of sister chromatid exchanges (SCEs) by styrene-7,8-oxide (SO) and 1,2-epoxy-3-butene (MEB), the epoxide metabolites of styrene and butadiene respectively and potential substrates for GSTM1. SCEs induced after a 48 h treatment (started 24 h after culture initiation) by two different concentrations of SO (50 and 150 μM) and MEB (50 and 250 μM) were analyzed in cultured (72 h) lymphocytes of six *GSTM1* null (gene deleted) and six *GSTM1*-positive (gene present) donors. Both SO and MEB were found to clearly increase SCEs. The *GSTM1* genotype had no influence on SCE induction by SO. In contrast, MEB produced a higher level of SCEs among the *GSTM1* null than *GSTM1*-positive samples. At 250 μM MEB, the *GSTM1* null donors showed 31% more induced SCEs (on average seven more SCEs per cell) than the *GSTM1*-positive donors ($P = 0.02$, acetone treatment as the reference). Furthermore, the *GSTM1* null genotype was associated with a slight decrease in mitotic index and replication index, regardless of the treatment. The results suggest that GSTM1-mediated GSH conjugation is an important detoxification pathway for MEB, but not for SO, in cultured human lymphocytes.**

The metabolism of carcinogenic compounds is modulated by several polymorphically expressed enzymes (1), including glutathione S-transferases (GST*; EC 2.5.1.18), a family of isoenzymes catalyzing the conjugation of reactive species to glutathione (GSH) (2). The polymorphic forms of glutathione S-transferase M1 (GSTM1) result from homo- and heterozygotic combinations of alleles *GSTM1/0*, *GSTM1/A* and *GSTM1/B* (1,3). Individuals with the *GSTM1 0/0* genotype, about 45% of Caucasians, lack the GSTM1 enzyme, being theoretically at a higher risk from the toxic effects of its substrates (4). The *GSTM1 0/0* genotype (i.e. *GSTM1* null genotype) has been considered to be one of the risk factors in developing different malignancies, including lung cancer (4–8).

*__Abbreviations:__ GST, glutathione S-transferase; GSH, glutathione; SCE, sister chromatid exchange; SO, styrene-7,8-oxide; MEB, 1,2-epoxy-3-butene; RI, replication index; MI, mitotic index; DEB, diepoxybutane.



The mean level of sister chromatid exchanges (SCEs) in peripheral lymphocytes has been shown to be higher among smokers lacking GSTM1 expression than among smokers who express GSTM1 (9), suggesting increased sensitivity to the genotoxic effects of smoking among individuals deficient for GSTM1. It has also been reported that GSTM1 deficiency is associated with increased sensitivity of cultured lymphocytes to SCE induction by *trans*-stilbene oxide, a model substrate for GSTM1, but not by *cis*-stilbene oxide, a poor substrate for the isozyme (10). The epoxide metabolites of styrene and 1,3-butadiene, styrene-7,8-oxide (SO) and 1,2-epoxy-3-butene (monoepoxybutene, MEB) respectively, are among the likely substrates for GSTM1 (11,12). Both SO and MEB have earlier been shown to be genotoxic in various assay systems (13–15) and are known inducers of SCEs in cultured human lymphocytes (16–20). The purpose of the present study was to examine whether the *GSTM1* genotype of lymphocyte donors influences the induction of SCEs by SO and MEB.

A 10 ml sample of heparinized peripheral blood was collected from 12 healthy volunteers, six having the *GSTM1* gene and six lacking it. The *GSTM1* genotype of each blood donor had earlier been determined by a PCR-based method described elsewhere (4). The individuals in the two groups were matched pairwise with respect to gender (three men and three women in both groups) and age (Table I) and were current non-smokers. From each person's blood sample, 12 whole-blood lymphocyte cultures were established, six for the MEB series and six for the SO series, in air-tight 20 ml glass injection bottles. A high and a low concentration of both chemicals was tested. In addition, cultures receiving only the solvent (acetone) or no treatment were set up as controls. The final concentrations, chosen according to earlier studies (15,16,18), were 250 and 50 μM for MEB and 150 and 50 μM for SO. SO and MEB (both 98% pure) were purchased from Aldrich Chemical Co. (Milwaukee, WI) and acetone from Merck (Darmstadt, Germany). Whole-blood lymphocyte cultures containing 6 ml growth medium and 0.3 ml peripheral blood were established as described earlier (19).

The cultures were exposed to the epoxides 24 h after initiation, using microsyringes (Hamilton Bonaduz AG, Switzerland). The volume added to each treated culture was 10 μl, the solvent control cultures receiving the same amount of acetone. Duplicate cultures were used from each donor for all treatments. The cells were harvested after 72 h of culture, as described earlier (19). The tubes containing the fixed cell suspensions were coded and air-dried microscopic slides were prepared and equipped with code numbers for blind analysis. The slides were stained using a modification of the fluorescence-plus-Giemsa technique (20) and were divided between two microscopists, one scoring the MEB series and the other the SO series; control cultures were analyzed by both scorers. For each culture, SCEs were scored in 25 second division cells, making a total of 50 cells per donor and treatment. In addition, the frequency of first, second and third division metaphases was evaluated from 100 cells/culture for replication

**Table I.** Mean no. SCEs/cell (50 cells scored per treatment and donor; SE between the means of two duplicate cultures shown in parentheses) after a 48 h *in vitro* treatment (started 24 h following culture initiation) with MEB in 72 h whole-blood lymphocyte cultures of donors genotyped as *GSTM1*⁻ or *GSTM1*⁺

| Genotype Donor | Gender | Age (years) | Untreated control | Acetone (solvent) | MEB (50 μM) | MEB (250 μM) |
|---|---|---|---|---|---|---|
| GSTM1⁻ | | | | | | |
| A | F | 25 | 7.5 (0.1) | 8.4 (0.8) | 12.5 (0.9) | 50.1 (0.8) |
| B | F | 44 | 9.0 (1.2) | 10.8 (0.7) | 14.5 (1.6) | 38.3 (1.2) |
| C | F | 46 | 10.6 (1.1) | 10.7 (1.2) | 12.8 (0.4) | 36.6 (7.0) |
| D | M | 36 | 7.8 (0.3) | 8.1 (0.2) | 12.2 (0.1) | 36.8 (7.3) |
| E | M | 34 | 8.0 (0.4) | 7.8 (0.4) | 15.7 (1.3) | 32.1 (6.9) |
| F | M | 28 | 6.9 (0.8) | 8.2 (0.3) | 11.7 (0.6) | 36.0 (4.6) |
| Mean (SD) | | 35.5 (8.4) | 8.3 (1.3) | 9.0 (1.4) | 13.2 (1.5) | 38.3[a] (6.1) |
| GSTM1⁺ | | | | | | |
| G | F | 27 | 8.6 (0.1) | 8.7 (0.8) | 12.9 (0.3) | 30.7 (1.0) |
| H | F | 40 | 8.7 (0.6) | 9.7 (0.1) | 12.5 (0.3) | 33.1 (1.7) |
| I | F | 44 | 5.7 (0.6) | 8.1 (0.1) | 10.8 (0.8) | 34.8 (3.6) |
| J | M | 46 | 10.2 (0.2) | 9.2 (0.9) | 13.2 (1.1) | 33.4 (1.7) |
| K | M | 38 | 7.6 (1.4) | 9.8 (0.8) | 11.9 (0.7) | 33.8 (4.7) |
| L | M | 32 | 7.6 (0.5) | 9.3 (0.0) | 10.8 (0.3) | 22.8 (8.7) |
| Mean (SD) | | 37.8 (7.2) | 8.1 (1.5) | 9.1 (0.6) | 12.1 (1.0) | 31.4 (4.9) |

[a] $P = 0.02$ for treatment×*GSTM1* genotype interaction, repeated measures analysis of variance, acetone treatment as the reference.

**Table II.** Mean no. SCEs/cell (50 cells scored per treatment and donor; SE between the means of two duplicate cultures shown in parentheses) after a 48 h *in vitro* treatment (started 24 h following culture initiation) with SO in 72 h whole-blood lymphocyte cultures of donors genotyped as *GSTM1*⁻ or *GSTM1*⁺

| Genotype Donor | Untreated control | Acetone (solvent) | SO (50 μM) | SO (150 μM) |
|---|---|---|---|---|
| GSTM1⁻ | | | | |
| A | 6.4 (0.2) | 7.9 (0.9) | 10.6 (0.3) | 21.8 (1.3) |
| B | 7.8 (0.5) | 7.5 (0.1) | 13.9 (0.1) | 25.6 (2.3)[a] |
| C | 9.1 (1.8) | 9.0 (0.3) | 12.0 (0.1) | 21.4 (0.2) |
| D | 6.4 (0.3) | 7.0 (0.0) | 9.4 (0.5) | 19.1 (1.5) |
| E | 6.7 (0.4) | 8.0 (0.8) | 11.3 (0.6) | 24.8 (3.4) |
| F | 7.0 (0.0) | 7.2 (0.4) | 11.0 (0.3) | 19.6 (0.2) |
| Mean (SD) | 7.2 (1.1) | 7.8 (0.7) | 11.4 (1.5) | 22.1 (2.9) |
| GSTM1⁺ | | | | |
| G | 7.7 (0.7) | 7.5 (0.8) | 11.2 (0.4) | 19.8 (0.4) |
| H | 7.3 (0.0) | 6.9 (0.5) | 12.9 (1.2) | 24.3 (1.8)[b] |
| I | 5.3 (0.2) | 6.4 (0.1) | 9.4 (1.0) | 21.5 (1.2) |
| J | 8.2 (0.4) | 9.1 (0.4) | 11.6 (1.1) | 20.0 (1.6) |
| K | 6.8 (0.3) | 6.9 (0.7) | 11.0 (0.2) | 22.6 (1.3) |
| L | 6.3 (0.1) | 7.7 (1.1) | 10.1 (0.1) | 18.9 (0.5) |
| Mean (SD) | 6.9 (1.0) | 7.4 (1.0) | 11.0 (1.1) | 21.2 (2.0) |

[a] 35 cells analyzed.
[b] 44 cells analyzed.

index (RI; mean no. of replications completed by the scored metaphases). Mitotic index (MI) was calculated as the percentage of mitotic cells among 1000 cells/culture. The two microscopists who independently scored the same untreated and acetone control slides (24 cultures) showed good agreement in their results on SCEs ($r = 0.72$, linear correlation coefficient), RI ($r = 0.81$) and MI ($r = 0.78$). The scorer responsible for the MEB series tended to record, on average, 1.3 more SCEs/cell than the other scorer.

The results were analyzed statistically by using repeated measures analysis of variance for the SCE data and the logistic binomial regression model for the RI and MI data (Egret software, revision 1; Statistical and Epidemiology Research Corporation and Cytel Software Corporation). In both of the statistical methods, the influence of the individual donor could be taken into account; for SCEs, this was accomplished by viewing each cell analyzed as a repeated measure. The *GSTM1* genotype was included in the statistical models both alone and as an interaction term genotype×treatment, the former reflecting the effect of genotype on the parameter in general and the latter in conjunction with exposure. Each epoxide concentration was analyzed separately, using acetone-treated controls as a reference. The models also included treatment, gender and replications as co-variables. Although SCEs/cell are not always normally distributed, the use of repeated analysis of variance was well justified in the present study, because residuals in the different models showed no deviation from normality in the Shapiro–Wilk test.

The results of the SCE analysis are presented in Table I for MEB and in Table II for SO. A significant increase ($P < 0.0001$) in SCEs was obtained with both concentrations of SO and MEB in cultures of all donors. Acetone also increased SCEs marginally ($P = 0.035$) in the MEB series, irrespective of donor genotype; in the SO series the difference in SCE frequencies between the untreated controls and the acetone treatment was not statistically significant.

A significant interaction between the *GSTM1* genotype and epoxide treatment was observed in SCE response only at the higher concentration of MEB (250 μM), the *GSTM1* null donors showing a mean no. of induced SCEs/cell (mean no. SCEs from each acetone-treated culture subtracted) that was 7 SCEs (31%) higher than that of the *GSTM1*-positive donors ($P = 0.02$, acetone-treated controls as the reference; $P = 0.002$, untreated + acetone-treated controls as the reference) (Figure 1). The lower concentration of MEB (50 μM) also showed a similar trend, with a mean difference of 1.3 induced SCEs/cell (45%) between the genotypes, which was not, however, statistically significant ($P = 0.081$, acetone-treated controls as the reference). In contrast, the *GSTM1* genotype had no influence on SCE induction by SO.

RI and MI were significantly ($P < 0.001$) decreased by the high doses of both epoxides; the low doses of MEB and SO slightly increased MI (Table III). Both RI and MI tended to be somewhat lower among the *GSTM1* null than *GSTM1*-positive donors, regardless of treatment. This finding suggests a basic difference in lymphocyte growth between the two groups, possibly related to their *GSTM1* genotype. No significant interaction between the epoxide treatment and *GSTM1* genotype could be seen for RI or MI when the acetone-treated controls were used as reference, suggesting that GSH

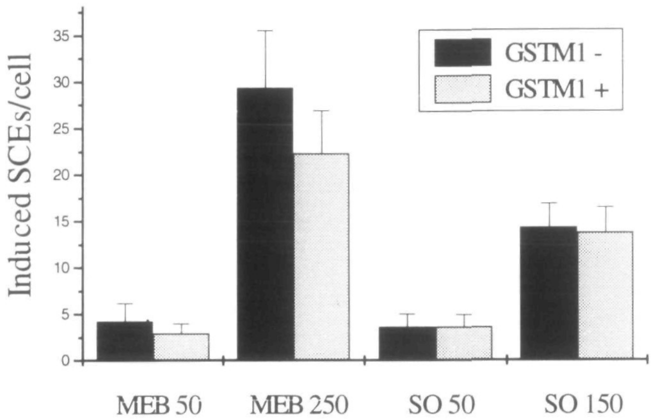


**Fig. 1.** Mean no. SCEs/cell (50 cells analyzed per donor and treatment) induced in 72 h human whole-blood lymphocyte cultures by MEB and SO after a 48 h *in vitro* treatment (started 24 h following culture initiation). The baseline SCE frequency (mean no. SCEs/cell from each acetone-treated culture) has been subtracted from the results. *GSTM1*$^+$, glutathione S-transferase M1-positive donors ($n = 6$); *GSTM1*$^-$, glutathione S-transferase M1 null donors ($n = 6$). Bars represent SD.

conjugation mediated by GSTM1 does not greatly modulate the toxicity (expressed as a decrease in RI or MI) of the two epoxides in cultured human lymphocytes.

The studies of Wiencke *et al.* (23,24), Kelsey *et al.* (21) and Wiencke and Kelsey (22) have earlier shown a bimodal distribution among blood donors in the sensitivity of their lymphocytes to the induction of SCEs by diepoxybutane (DEB), another metabolite of 1,3-butadiene. The sensitive donors represented about 20% of 173 individuals tested (22). In a subset of four sensitive and six insensitive donors, three (75%) and two (33%) respectively were GSTM1-deficient. The mean no. SCEs/cell (at a 6 μM concentration of DEB) was about 23 SCEs higher among the five GSTM1-deficient compared with the five GSTM1-non-deficient donors. Wiencke *et al.* (23) concluded, however, that GSTM1 deficiency is not responsible for the bimodal distribution of DEB-induced SCEs, although they could not rule out a subtle effect (22,23). Individuals sensitive to DEB also showed high rates of DEB-induced chromatid aberrations, elevated baseline SCE frequencies and an increased SCE response after an *in vitro* treatment with MEB (22). Possibly because of the open cultures used (MEB is fairly volatile at 37°C), Wiencke and Kelsey (22) required a four times higher nominal concentration of MEB to obtain roughly similar increases in SCE levels as we observed in closed cultures (1 mM versus 250 μM). At a 1 mM concentration of MEB, the difference in the mean no. SCEs/cell between the three sensitive and six resistant donors was 8.6 SCEs; information about the GSTM1 phenotype of these donors was not available (22). Thus, the existing data would indicate that the *in vitro* cytogenetic effects of the epoxide metabolites of 1,3-butadiene are modulated by at least two traits: the DEB-sensitive phenotype and the *GSTM1* genotype. It is still unclear to what extent these characteristics are related to each other and if other known polymorphic phase II enzymes, such as GSTθ, play a role in determining individual sensitivity to these epoxides.

The present results suggest that, under the experimental conditions applied, GSTM1-mediated conjugation to GSH is a significant detoxification pathway for MEB, but not for SO. This interpretation agrees with the known metabolism of styrene in humans, where GSH conjugation represents only a minor detoxification pathway, the reaction catalyzed by epoxide hydrolase being more important (15). Epoxide hydrolase-mediated hydrolysis appears to be effective in detoxifying MEB



**Table III.** Mean RI (mean no. replications completed by the 200 metaphases scored per donor and treatment) and MI (percentage mitotic cells in 2000 cells per donor and treatment) in 72 h human whole-blood lymphocyte cultures of *GSTM1*$^+$ ($n = 6$) and *GSTM1*$^-$ donors ($n = 6$) after a 48 h *in vitro* treatment (started 24 h following culture initiation) with MEB or SO

| Treatment (μM) | Mean RI (SD) | | Mean MI (SD) | |
|---|---|---|---|---|
| | GSTM1$^+$ | GSTM1$^-$ | GSTM1$^+$ | GSTM1$^-$ |
| MEB | | | | |
| 0 (untreated) | 2.4 (0.1) | 2.4 (0.2) | 5.9 (1.8) | 5.5 (1.3) |
| 0 (acetone) | 2.4 (0.1) | 2.3 (0.2) | 5.7 (1.6) | 4.9 (2.1) |
| 50 | 2.5 (0.1) | 2.4 (0.1) | 7.6 (0.9) | 6.6 (1.9) |
| 250 | 2.1 (0.2) | 1.9 (0.1) | 2.5 (0.5) | 2.5 (0.9) |
| SO | | | | |
| 0 (untreated) | 2.5 (0.2) | 2.3 (0.2) | 5.3 (1.4) | 4.9 (1.5) |
| 0 (acetone) | 2.5 (0.1) | 2.3 (0.1) | 4.5 (0.9) | 4.0 (0.8) |
| 50 | 2.5 (0.1) | 2.4 (0.2) | 6.8 (2.5) | 5.5 (1.7) |
| 150 | 2.0 (0.2) | 1.8 (0.1) | 2.0 (0.4) | 1.5 (1.1) |

in human liver fractions as well, although GSH conjugation also plays a role (25).

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