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Glutathione transferase μ deficiency is not a marker for predisposition to sulphonamide toxicity

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The mechanism(s) by which sulphonamides cause adverse reactions has been an area of extensive research which has yet to provide many definitive answers. Previous *in vitro* studies conducted in this laboratory have suggested that glutathione (GSH) and/or its metabolizing enzymes may play a pivotal role in detoxifying reactive metabolites of various sulphonamides: glutathione and *N*-acetyl cysteine decreased both the irreversible binding [1] and toxicity [1–3] of metabolites of sulphadiazine and sulphamethoxazole; cells (peripheral blood mononuclear cells; PBMC) from individuals with glutathione synthetase deficiency were more sensitive to toxic metabolites generated from sulphonamides than were cells from healthy volunteers [1]. From this work, it was postulated that an inherited deficiency in glutathione-*S*-transferase (GST) activity within the target cells was responsible for the development of sulphonamide toxicity in susceptible individuals [1, 3].

The considerable inter-individual variation in a particular isozyme of the GST superfamily, GSTμ [4], has prompted investigations aimed at identifying whether this variation could be responsible for susceptibility to drugs and toxins. Recent evidence suggests that an inherited deficiency in this enzyme may be a marker for susceptibility to lung cancer among smokers [5, 6] and chemically-induced cytogenetic damage [7]. Experiments have shown that GSTμ present in PBMC is identical to that expressed in the liver [8] and that GSTμ polymorphism exists in a wide range of tissues frequently implicated in sulphonamide hypersensitivity reactions [9]. In theory, therefore, although unlikely to be responsible *per se*, GSTμ deficiency may contribute to the predisposition of certain individuals to sulphonamide toxicity. The aim of the present study was to test this hypothesis by examining whether GSTμ activity in PBMC correlated with the *in vitro* toxicity of reactive metabolites of sulphamethoxazole towards these target cells in healthy volunteers and patients who had experienced adverse reactions to sulphonamides.

Materials and Methods

In vitro *toxicity assay*. The *in vitro* assay for metabolite-induced toxicity has been previously reported in detail [10]. Ethical approval for the studies was obtained from the Scientific and Ethics Review Committees of the Hospital for Sick Children. PBMC were prepared from whole blood of 12 patients (aged 2–39 years) with suspected hypersensitivity reactions to sulphonamides and 13 healthy volunteers (aged 20–46 years) with Ficoll-paque and suspended in a HEPES-buffered medium to give 1×10^6 cells per reaction. Hepatic microsomes were prepared from National Institutes of Health General Purpose Swiss Mice [N: GP(SW)] pretreated with phenobarbitone (60 mg/kg body wt, i.p. for 3 days). Microsomal protein (0.3 mg) was incubated with PBMC at 37° for 2 hr along with an NADPH-generating system (0.6 mM NADP, 2.4 mM glucose-6-phosphate, 2 units of glucose-6-phosphate dehydrogenase) and drug (2 mM sulphamethoxazole; SMX). The drug was added in dimethyl sulphoxide (final concentration 2.5%) which was not associated with any toxicity. Cells were collected by centrifugation and resuspended in HEPES-buffered medium containing 5 mg/mL albumin. Incubations were continued at 37° for 16 hr and aliquots were then taken for viability assessment by trypan blue exclusion. At least 200 cells were examined per sample in a blind protocol.

Determination of glutathione-S-transferase μ activity. The procedure was essentially that described by Seidegard *et al.* [11]. Briefly, 3×10^6 PBMC, disrupted by sonication, were incubated for 4 min at 37° in 175 mM sodium phosphate buffer (pH 7.2) containing 5 mM reduced glutathione and 250 μM (0.3 μCi) [^{3}H]*trans*-stilbene oxide (sp. act. 1.4 Ci/mmol, >97% pure; Chemsyn Science Laboratories, Lenexa, KA, U.S.A.). The total incubation volume was 100 μL. The reaction was terminated by extraction (×2) with 200 μL *n*-hexyl alcohol. The radioactive glutathione conjugates remaining in the aqueous phase were then quantified (pmol/min/10^7 cells) by liquid scintillation spectrometry.

Results

Figure 1 shows the toxicity manifested by sulphamethoxazole metabolites towards peripheral blood mononuclear cells from healthy volunteers and patients which had reported with sulphonamide toxicity. Significant ($P < 0.001$) metabolite-mediated toxicity was only observed towards cells obtained from individuals suspected of experiencing an adverse reaction to a sulphonamide(s), although the total glutathione content (0.79 ± 0.06 nmol/10^6 cells vs 0.76 ± 0.06 nmol/10^6 cells) and glutathione reductase activity (1.12 ± 0.07 mUnits/10^6 cells vs

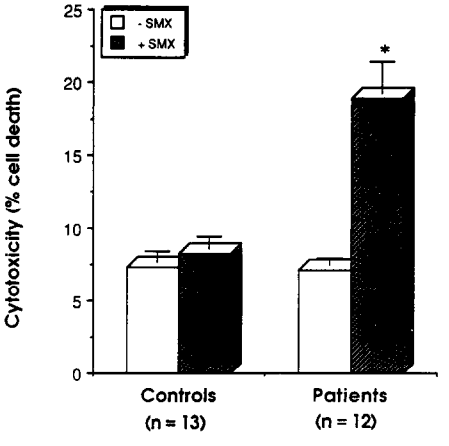

Fig. 1. Toxicity of sulphamethoxazole (SMX) metabolites towards peripheral blood mononuclear cells from patients who had experienced hypersensitivity reactions to sulphonamides and from healthy volunteers (some of which had tolerated sulphonamide therapy). Data are expressed as mean ± SEM. For each subject, mean values were obtained from one to three experiments performed in triplicate. *Significantly different from values obtained in incubations conducted in absence of sulphamethoxazole, $P < 0.001$ (Wilcoxon signed rank test).

0.96 ± 0.08 mUnits/10^6 cells) did not differ significantly between the two groups. Indeed, cells from 11 of the 12 patients examined (92%) were susceptible to toxicity mediated by SMX metabolites compared with none of the PBMC samples taken from normal individuals. For patients, cytotoxicity was enhanced from 7.1 ± 0.5% (mean ± SEM) to 18.9 ± 2.1% in incubations conducted in the presence of sulphamethoxazole (2 mM) and metabolite-mediated toxicity (11.8%) was significantly greater than for controls (0.9%; $P < 0.001$).

Measurement of GSTμ activity in PBMC revealed that there was a large inter-individual variation (100–200-fold) in the activity of this detoxifying enzyme (Fig. 2A), as previously reported [11]. GSTμ activity was similar in both populations ($P > 0.05$, Wilcoxon test), indicating that individuals deficient in GSTμ (sp. act. < 100 pmol/min/10^7 cells) [11] were no more susceptible to sulphonamide toxicity than subjects with moderate to high activity. Indeed, further analysis showed there was no direct relationship between GSTμ activity measured in PBMC and the susceptibility of these cells to toxicity mediated by sulphonamide metabolites ($r^2 = 0.119$, $P = 0.09$; Fig. 2B).

Discussion

Drug toxicity is largely governed by a delicate balance between activating and detoxifying biotransformations [12–14]. It has been proposed that 'slow acetylators' may be predisposed to the adverse effects of sulphonamides as more drug is available in these individuals for activation via oxidative metabolism. However, although N-acetylation undoubtedly constitutes an important route of detoxication for some sulphonamides [15], half the population are slow acetylators. Therefore, although previous studies have demonstrated that there is a preponderance of slow acetylators among people who experience hypersensitivity reactions to sulphonamides [2], this metabolic defect cannot solely account for the low incidence (<1%) of these life-threatening reactions. Predisposition to sulphonamide toxicity may therefore be determined by multiple factors, including enhanced metabolic activation of the parent amine by a particular isozyme(s) of cytochrome P450, decreased detoxication by *N*-acetyl transferase and some other drug detoxification defect.

A critical step in the bioactivation of sulphonamides is oxidation of the amine to the hydroxylamine [16, 17]. These metabolites possess limited chemical reactivity and, hence, a further reaction must occur to yield the ultimate toxin: oxidation to the highly reactive nitroso compound [18] or possibly conjugation of the hydroxylamine (acetylation, glucuronidation or sulphation), which may result in reactive nitrenium or carbonium ions. Studies with nitrosobenzene [19], nitrosochloramphenicol [20] and carcinogenic aromatic amines [21] have shown that such reactive metabolites may be detoxified through conjugation with glutathione. Although these intermediates may react spontaneously with glutathione, experiments with *N*-hydroxyarylamines derived from foodstuffs have shown that the non-enzymatic conjugation of these less reactive compounds is slow and that GST can catalyse this reaction [21]. However, relatively little is known about the isozyme specificity of such biotransformations, particularly with reference to human GST. Recent data obtained in this laboratory (which will be published elsewhere) support the hypothesis that reactive metabolites of sulphonamides may also undergo conjugation with this ubiquitous tripeptide.

Preliminary studies showed that GST activity towards the prototypic substrate 1-chloro-2,4-dinitrobenzene is similar in patients who experience adverse reactions to sulphonamides and healthy controls (Spielberg, unpublished data) suggesting that there is no general deficiency in GST activity amongst individuals predisposed to sulphonamide toxicity. Hence, any relevant inter-individual differences in GST activity must be related to a particular isozyme. The present study, albeit limited in power by the rarity of the adverse reactions and, consequently, available subjects, demonstrates that GSTμ deficiency, a metabolic defect associated with other forms of chemically induced toxicity [5, 7], does not contribute to the manifestation of sulphonamide toxicity. Cells from subjects deficient in GSTμ were no more susceptible to the

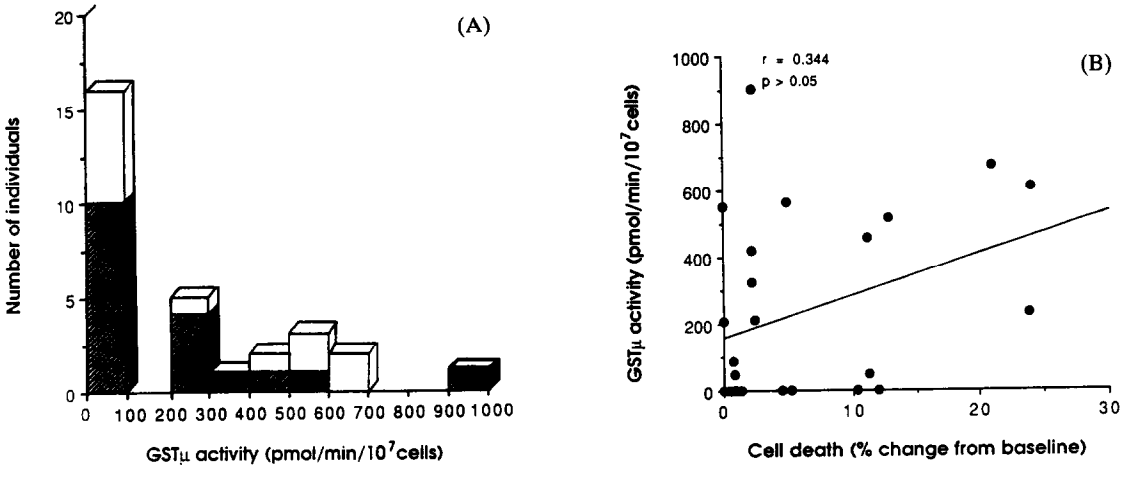

Fig. 2. (A) Distribution of glutathione-*S*-transferase μ activity in peripheral blood mononuclear cells from patients (open bars) and healthy volunteers (hatched bars). For each individual, activity is expressed as the mean of one or two experiments performed in triplicate. There was no difference between activities measured in patients' cells and those assessed in cells from volunteers ($P > 0.05$, Wilcoxon test for unpaired data). (B) Relationship between glutathione-*S*-transferase activity measured in peripheral blood mononuclear cells and the susceptibility of these cells to sulphamethoxazole metabolite-mediated toxicity. The correlation coefficient *r* was determined by least squares regression analysis. Baseline toxicity refers to that observed with vehicle alone (see Materials and Methods).

toxicity mediated by reactive metabolites of sulphamethoxazole than cells from subjects with moderate or high GSTμ activity. Furthermore, the distribution of GSTμ activity within a population of normal healthy volunteers (N = 18) was not different from the distribution in a group of individuals (N = 12) who had experienced adverse reactions to sulphonamides (Fig. 2A) and there was no correlation between GSTμ activity and the susceptibility of PBMC to toxicity mediated by sulphamethoxazole metabolites ($r^2 = 0.119$, $P > 0.05$; Fig. 2B).

In summary, glutathione transferase μ activity, a marker for susceptibility to lung cancer and chemically induced cytogenetic damage [5–7], is not a predictive index for the predisposition to sulphonamide hypersensitivity reactions. However, considering the functional diversity and broad, overlapping substrate specificity of GSH-dependent enzymes, it is conceivable that an as yet unidentified deficiency in another GST isozyme or GSH-related enzyme may be a marker for sulphonamide toxicity. In addition, heterogeneity in cellular repair mechanisms and the diversity of the human immune response [22] may also contribute to the manifestation of the toxic effects of sulphonamides. Experiments are currently in progress to determine which of this myriad of variables is predominantly responsible for inter-individual susceptibility to the idiosyncratic reactions produced by these antibacterial agents.

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