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HUMAN ERYTHROCYTE GLUTATHIONE S-TRANSFERASE: A POSSIBLE MARKER OF CHEMICAL EXPOSURE

(Biological marker; chemical exposure; industrial toxicants; acrolein; epoxides; 1,2-dihaloethanes)

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SUMMARY

We have explored the possibility of using glutathione S-transferase (GST) as a biological marker of chemical exposure. All the model compounds tested in the present study (acrolein, propylene oxide, styrene oxide, ethylene dibromide and ethylene dichloride) showed a dose-dependent inactivation of erythrocyte GST in situ as well as the inhibition of purified erythrocyte GST.

INTRODUCTION

Many compounds of industrial interest and/or their metabolites are toxic and possess an electrophilic center which can react with cellular nucleophiles such as glutathione (GSH). These electrophilic compounds form thioethers upon interaction with GSH and this reaction is catalyzed by a family of enzymes known as glutathione S-transferase (EC 2.5.1.18) [1–3]. Glutathione S-transferase (GST) can catalyze the conjugation of compounds having an electrophilic center to GSH [1,2], remove toxic compounds from circulation through non-catalytic binding [3], and reduce lipid hydroperoxides through their GSH peroxidase II activity [4,5]. Most of

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Abbreviations: GST, glutathione S-transferase; GSH, glutathione; PBS, phosphate buffer saline; BSP, bromosulfophthalein; CDNB, 1-chloro-2,4-dinitrobenzene.

the mammalian tissues examined so far contain multiple forms of GST [6,7].

In recent years a number of compounds have been shown to inhibit GST isoenzymes of human and rat tissues in vitro and in vivo [8–11]. During the present investigation we have studied the effect of some of the toxic industrial chemicals on GST of human erythrocytes in situ as well as on purified GST isoenzyme from human erythrocytes and explored the possibility of using GST activity of erythrocytes as a marker of chemical exposure.

Results of these studies indicate that these chemicals (acrolein, styrene oxide, propylene oxide, ethylene dibromide and ethylene dichloride) inactivate the GST of human erythrocytes in situ as well as inhibit the purified anionic GST of human erythrocytes in vitro and, therefore, the extent of inhibition of GST activity of human erythrocytes may be used as a measure of chemical exposure to these compounds.

MATERIALS AND METHODS

Acrolein (97%), styrene oxide (97%), propylene oxide (99%), ethylene dibromide (99%), and ethylene dichloride (99%) were purchased from Aldrich Chemical Company (Milwaukee, WI). Sources of other chemicals used in this study have been described previously by us [12].

Purification of GST of human erythrocytes

The GST from human erythrocytes was purified according to the scheme described by us previously [12]. Briefly, blood was centrifuged at 2000 rpm to remove plasma and buffy coat followed by filtration over cotton wool to remove leukocytes. The erythrocytes were washed thoroughly with phosphate-buffered saline (10 mM potassium phosphate, pH 7.0, containing 150 mM sodium chloride; PBS). Erythrocytes were hemolyzed by mixing with 9 volumes of potassium phosphate (5 mM, pH 7.0) containing 1.4 mM 2-mercaptoethanol (buffer A) and dialyzed against 22 mM potassium phosphate (pH 7.0) containing 1.4 mM 2-mercaptoethanol (buffer B). After centrifugation at 10000 $\times$ g for 1 h, the supernatant was passed through a GSH-linked epoxy-activated Sepharose 6B column [13] and the column was washed with buffer B and then eluted with 5 mM GSH in 50 mM Tris-HCl (pH 9.6) containing 1.4 mM 2-mercaptoethanol. After dialysis against 10 mM potassium phosphate buffer (pH 7.4) the enzyme was passed through a column of bromosulfophthalein (BSP) bound to S-glutathione agarose [14]. GST activity was determined according to the method of Habig et al. [15]. About 96% of the total GST activity applied on the BSP-GSH-agarose affinity column appeared in the unabsorbed fraction as a sharp peak and the isoelectric point of the isoenzyme was found to be 4.5. The remaining activity was eluted as a single sharp peak by 10 mM GSH in 50 mM Tris-HCl buffer, pH 9.6, containing 1.4 mM 2-mercaptoethanol and the pI of the isoenzyme was found to be $>$ 10. The substrate specificities, im-

munological properties and structural properties of the cationic isoenzyme, GST σ (pI > 10), and the anionic isoenzyme, GST ϱ (pI 4.5), purified from human erythrocytes during the present study were similar to those described by us previously for these isoenzymes [12]. Since the acidic isoenzyme ϱ accounts for more than 95% of the GST activity of erythrocytes towards CDNB, the inhibitory effect of the compounds used in the present investigation was studied only on this isoenzyme. Prior to the inhibition studies, GST ϱ was dialyzed against buffer A and the dialyzed enzyme showed the presence of a single polypeptide band (M_r 22 500) in urea/SDS/polyacrylamide slab gel electrophoretograms [16] indicating apparent homogeneity of the enzyme.

Inhibition studies

The inhibition studies were performed at pH 6.5 with 1-chloro-2,4-dinitrobenzene (CDNB) as the electrophilic substrate. Appropriate amounts of chemical dissolved in 50 μl ethanol were added to the enzyme solution (20 μl, containing approximately 120 ng protein, specific activity = 40) and the activity was determined using 1 mM GSH and 1 mM CDNB in 100 mM potassium phosphate buffer (pH 6.5) essentially according to the method of Habig et al. [15]. One unit of enzyme utilized 1 μmol substrate (CDNB)/min at 25°C.

The effect of chemicals was also studied on GST activity of erythrocytes in situ. Fresh blood from normal healthy donors was centrifuged at 2000 rpm and plasma and buffy coats were removed. Red cells were washed 3 times with PBS, suspended in 3 volumes of PBS and incubated with desired amounts of the chemical in a total volume of 1 ml at 25°C for 1 h. Following the incubation, erythrocytes were hemolyzed with buffer A, centrifuged at 14 000 × g for 20 min, and GST activity towards CDNB was determined in the supernatant. The control samples were processed under similar conditions, except that the inhibitors were omitted during the incubations.

RESULTS AND DISCUSSION

The results of studies on the inhibition of anionic GST ϱ [17] of erythrocytes (pI 4.5) by acrolein, propylene oxide, styrene oxide, ethylene dibromide and ethylene dichloride are presented in Fig. 1. GST ϱ was inhibited by all these compounds and acrolein was found to be comparatively more inhibitory among all these compounds. All the chemicals studied showed a dose-dependent inhibition with I_{50} being in the range of 10^{-3} M to 10^{-4} M. With acrolein, a maximum inhibition of 75% was observed at 1×10^{-3} M concentration and the I_{50} was found to be 5.7×10^{-4} M (Fig. 1A). With propylene oxide, maximum inhibition of 60% was observed at 5×10^{-3} M concentration with an I_{50} value of 3.1×10^{-3} M (Fig. 1B). Maximum inhibition of 57% was observed with styrene oxide at 5×10^{-3} M concentration and the I_{50} value was found to be 4.2×10^{-3} M (Fig. 1B). Maximum inhibition

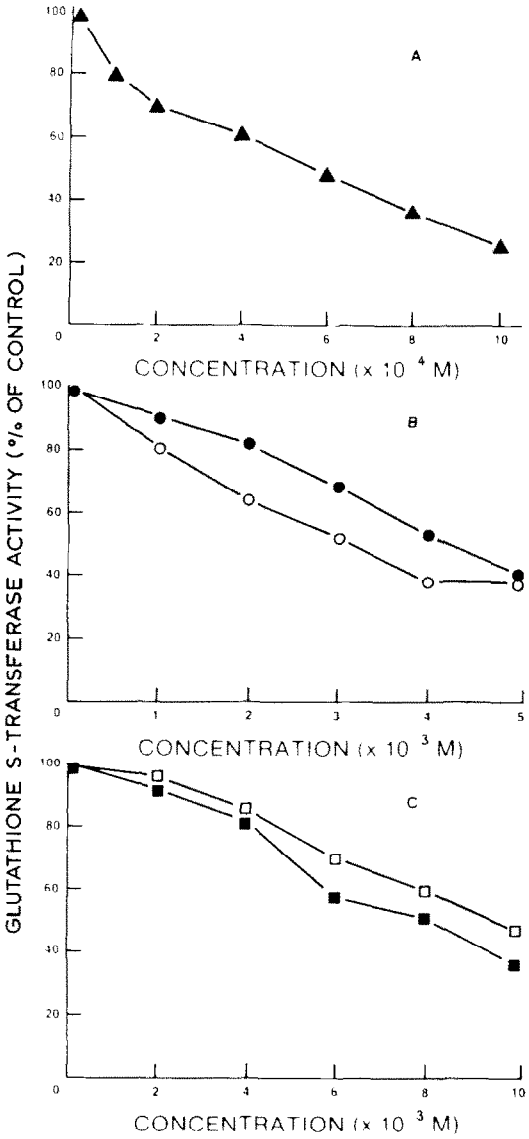

Fig. 1. Inhibition of purified anionic GST of human erythrocytes by (*A*) ▲—▲, acrolein; (*B*) ○—○, propylene oxide; ●—●, styrene oxide; and (*C*) ■—■, ethylene dibromide; and □—□, ethylene dichloride. The zero concentration assays were performed in the presence of 50 μl ethanol.

of 60% was observed by ethylene dibromide at 10×10^{-3} M concentration and the maximum inhibition observed by ethylene dichloride was only 50% at this concentration, 10×10^{-3} M (Fig. 1C). The I_{50} values for inhibition of GST of human erythrocytes by ethylene dibromide and ethylene dichloride were found to be 8.2×10^{-3} M and 10×10^{-3} M, respectively. Due to the low enzyme activity to cationic

TABLE I

EFFECT OF ACROLEIN, PROPYLENE OXIDE, STYRENE OXIDE, ETHYLENE DIBROMIDE AND ETHYLENE DICHLORIDE ON GST ACTIVITY OF HUMAN ERYTHROCYTES IN SITU

The experiment was performed three times and a representative set of data from one experiment is presented.

Chemical	Concentration (M)	GST activity (% of control)
Acrolein	2.8×10^{-4}	80.0
	5.7×10^{-4}	54.0
	8.5×10^{-4}	23.5
Propylene oxide	1.5×10^{-3}	71.5
	3.0×10^{-3}	57.4
	4.5×10^{-3}	22.7
Styrene oxide	2.0×10^{-3}	44.0
	4.0×10^{-3}	32.4
	6.0×10^{-3}	15.0
Ethylene dibromide	3.0×10^{-3}	77.2
	6.0×10^{-3}	76.5
	9.0×10^{-3}	50.0
Ethylene dichloride	3.0×10^{-3}	65.7
	6.0×10^{-3}	58.2
	9.0×10^{-3}	48.8

GST of erythrocytes (GST σ), the effect of these compounds coult not be studied on this isoenzyme.

Table I records the effect of these chemicals on GST activity of erythrocytes in situ. As can be seen in Table I, these compounds inhibited the GST activity in a dose-dependent manner and the I_{50} values were found to be in close agreement with the I_{50} values determined with purified anionic GST isoenzyme.

We have evaluated the possibility of using GST activity level in human erythrocytes as a biological marker of chemical exposure of certain industrial toxicants. All the chemicals used in this study inhibit purified GST ϱ and GST activity in situ. This suggests that chemical exposure will result in the reduced capability of erythrocyte GST to detoxify the xenobiotics. The I_{50} values of some of the chemicals used in this study are relatively high. It is not known if the concentrations of these compounds in the blood of chronically exposed industrial workers reach these levels. Further studies are, therefore, needed to evaluate the usefulness of the inhibition of erythrocyte GST by these compounds. However, detailed population studies on industrial sites need to be performed before GST is eventually used as a marker of chemical exposure to industrial workers.

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