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MOLECULAR PHARMACOLOGY, **57:**619–624 (2000).

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The Human Glutathione Transferase P1-1 Specific Inhibitor TER 117 Designed for Overcoming Cytostatic-Drug Resistance Is also a Strong Inhibitor of Glyoxalase I

ANN-SOFIE JOHANSSON, MARIANNE RIDDERSTRÖM, and BENGT MANNERVIK

Department of Biochemistry, Uppsala University, Biomedical Center, Uppsala, Sweden

Received July 13, 1999; accepted November 12, 1999

This paper is available online at http://www.molpharm.org

ABSTRACT

γ-L-Glutamyl-*S*-(benzyl)-L-cysteinyl-*R*-(−)-phenylglycine (TER 117) has previously been developed for selective inhibition of human glutathione *S*-transferase P1-1 (GST P1-1) based on the postulated contribution of this isoenzyme to the development of drug resistance in cancer cells. In the present investigation, the inhibitory effect of TER 117 on the human glyoxalase system was studied. Although designed as an inhibitor specific for GST P1-1, TER 117 also competitively inhibits glyoxalase I (K_I = 0.56 μM). In contrast, no inhibition of glyoxalase II was detected. Reduced glyoxalase activity is expected to raise intracellular levels of toxic 2-oxoaldehydes otherwise eliminated by glyoxalase I. The resulting toxicity would accompany the potentiation of cytostatic drugs, caused by inhibition of the detoxication effected by GST P1-1. TER 117 was designed for efficient inhibition of the most abundant form GST P1-1/Ile105. Therefore, the inhibitory effect of TER 117 on a second allelic variant GST P1-1/Val105 was also studied. TER 117 was shown to competitively inhibit both GST P1-1 variants. The apparent K_I values at glutathione concentrations relevant to the intracellular milieu were in the micromolar range for both enzyme forms. Extrapolation to free enzyme produced K_I values of approximately 0.1 μM for both isoenzymes, reflecting the high affinity of GST P1-1 for the inhibitor. Thus, the allelic variation in position 105 of GST P1-1 does not affect the inhibitory potency of TER 117. The inhibitory effects of TER 117 on GST P1-1 and glyoxalase I activities may act in synergy in the cell and improve the effectiveness of chemotherapy.

A major problem in the treatment of cancer with chemotherapy is the development of resistance of the cancer cells toward the cytostatic drugs used. The resistance is in part caused by an increased metabolic detoxication of the drugs in the cancer cells. A common feature of most mammalian tumor forms is elevated levels of glutathione transferase P1-1 relative to their concentration in the corresponding normal tissue (Mannervik et al., 1987; Moscow et al., 1989; Castro et al., 1990; Tsuchida and Sato, 1992). The glutathione *S*-transferases (GSTs; EC 2.5.1.18), comprise a family of widely distributed phase II detoxication enzymes that catalyze the conjugation of a broad variety of reactive electrophiles to the nucleophilic sulfur atom of the major intracellular thiol, the tripeptide glutathione [γ-L-glutamyl-L-cysteinylglycine (GSH); Fig. 1a]. The soluble GSTs are divided into distinct classes based on similarities in their primary structures and substrate specificities (Mannervik et al., 1985).

GST P1-1 has been shown to catalyze the conjugation of GSH with the alkylating agents chlorambucil (Ciaccio et al., 1990) and thiotepa (Dirven et al., 1995), suggesting that overexpression of GST P1-1 in cells exposed to these drugs would confer resistance. Elevated cellular levels of GST P1-1 have been shown to accompany resistance to various common anticancer drugs (Whelan et al., 1992; O'Brien and Tew, 1996), and the addition of the GST inhibitor ethacrynic acid restored sensitivity to alkylating agents in drug-resistant cells (Tew et al., 1988; Hansson et al., 1991). Furthermore, transfection of GST Pi antisense cDNA increased the sensitivity of a cancer cell line to various anticancer drugs (Ban et al., 1996). Increased levels of GST P1-1 mRNA in cells resistant to ethacrynic acid, not only an inhibitor but also a substrate for GST P1-1, have been reported (Kuzmich et al., 1992). Introduction of GST P1-1 into various cells in culture (Berhane et al., 1994; Tew, 1994; Cnubben et al., 1998) has been shown to protect against the cytotoxic effects of several drugs.

To circumvent the indicated contribution of GST P1-1 to drug resistance of tumor cells, an inhibitor, TER 117, that specifically inhibits the GST P1-1 isoenzyme was developed (Lyttle et al., 1994). This inhibitor, γ-L-glutamyl-*S*-(benzyl)-L-cysteinyl-*R*-(−)-phenylglycine (Fig. 1c), is a GSH analog

This work was supported by the Swedish Cancer Society. A.-S.J. is a recipient of a stipend from the Lilly and Sven Lawski Fund.

ABBREVIATIONS: GST, glutathione *S*-transferase; CDNB, 1-chloro-2,4-dinitrobenzene; GSH, glutathione; DEE, diethylester; TER 117, γ-L-glutamyl-(*S*-benzyl)-L-cysteinyl-D-phenylglycine; MG-SG, the hemithioacetal formed between glutathione and methylglyoxal.

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designed to block the active site of GST P1-1. To facilitate the cellular uptake of TER 117, it is delivered as a diethyl ester (TER 117 DEE, also called TER 199). In the cell, deesterification releases the active inhibitor. The treatment of drug-resistant cell lines with TER 117 DEE renders them sensitive to anticancer drugs such as chlorambucil and melphalan (Morgan et al., 1996).

Human GST P1-1 is polymorphic, and four allelic variants, Ile-105/Ala-114 (Board et al., 1989), Ile-105/Val-114 (Watson et al., 1998), Val-105/Ala-114 (Ahmad et al., 1990), and Val-105/Val-114 (Ali-Osman et al., 1997), have been identified. [The numbering of amino acid residues includes the initiator methionine as number 1 in agreement with the numbering of codons (cf. Mannervik and Widersten, 1995); the site of allelic variation is thus at residue 105. Some publications refer to this position as 104. The allelic variants studied, GST P1-1/Val-105 and GST P1-1/Ile-105, both contain Ala in position 114.] GST P1-1/Val-105 and GST P1-1/Ile-105 were originally purified from human placenta (Ahmad et al., 1990), but GST P1-1/Val-105 was subsequently also found in the tumor cell line HeLa in our laboratory (X.-Y. Hao and B.M., unpublished data). Residue 105 contributes to the hydrophobic substrate-binding site as evidenced by the crystal structure, whereas residue 114 is near the surface of the protein molecule outside the active site (Reinemer et al., 1992). The inhibitor TER 117 was developed for efficient inhibition using assays of the GST P1-1/Ile-105 isoenzyme. Because amino acid substitutions in position 105 have been shown to affect the substrate specificity of the enzyme (Zimniak et al., 1994; Ali-Osman et al., 1997; Johansson et al., 1998; Sundberg et al., 1998), the replacement of Ile with Val in this position might influence the affinity for TER 117 and consequently its inhibitory efficacy. Therefore, comparative inhibition studies including the two allelic variants GST P1-1/Ile-105 and GST P1-1/Val-105 were performed in the present study. Furthermore, because the inhibitory effect of TER 117 has so far been investigated only with GSTs, the possible effect of this inhibitor on the glyoxalase system was studied.

The glyoxalase system catalyzes the conversion of 2-oxoaldehydes into the corresponding 2-hydroxycarboxylic acids using GSH as coenzyme. The system is composed of glyoxalase I (EC 4.4.1.5) and glyoxalase II (EC 3.1.2.6) catalyzing consecutive reactions (Mannervik, 1980; Vander Jagt, 1989; Thornalley, 1993). Glyoxalase I catalyzes the isomerization of the hemithioacetal formed spontaneously between GSH and 2-oxoaldehydes. The resulting thiolester is a substrate for glyoxalase II, which catalyzes its hydrolysis into GSH and free 2-hydroxycarboxylic acid. An endogenous glyoxalase I substrate appears to be the cytotoxic methylglyoxal, a by-product of glycolysis. The glyoxalase system is thus considered to be involved in detoxication (Mannervik, 1980; Thornalley, 1993). Glyoxalase I, like GST P1-1, is frequently overexpressed in malignant tissues and tumor cell lines compared with corresponding normal levels (Thornalley, 1995). Raised glyoxalase I activity may be a consequence of increased glycolytic activity in cancer cells and provides a rationale for the development of antitumor agents. Inhibition of glyoxalase I may be expected to raise methylglyoxal concentrations to toxic levels (Vince and Daluge, 1971), and attempts to target tumor cells with such glyoxalase I inhibitors have been performed (Thornalley, 1995; Thornalley et al., 1996; Kavarana et al., 1999).

Materials and Methods

All chemicals used were of highest purity available. TER 117 and TER 117 DEE (TER 199) were provided by Telik, Inc., formerly Terrapin Technologies, Inc. (South San Fransisco, CA). Methylglyoxal was prepared as described by Kellum et al. (1978). *S*-D-Lactoylglutathione was synthesized enzymatically and purified as previously described (Uotila, 1981).

Enzymes. The enzymes used were recombinant proteins obtained by heterologous expression in *Escherichia coli*. In all cases, their catalytic properties have been shown to be indistinguishable from those of the enzymes isolated from human tissues. The GST P1-1 variants were expressed and purified essentially as previously described (Johansson et al., 1998). Human glyoxalase I and human

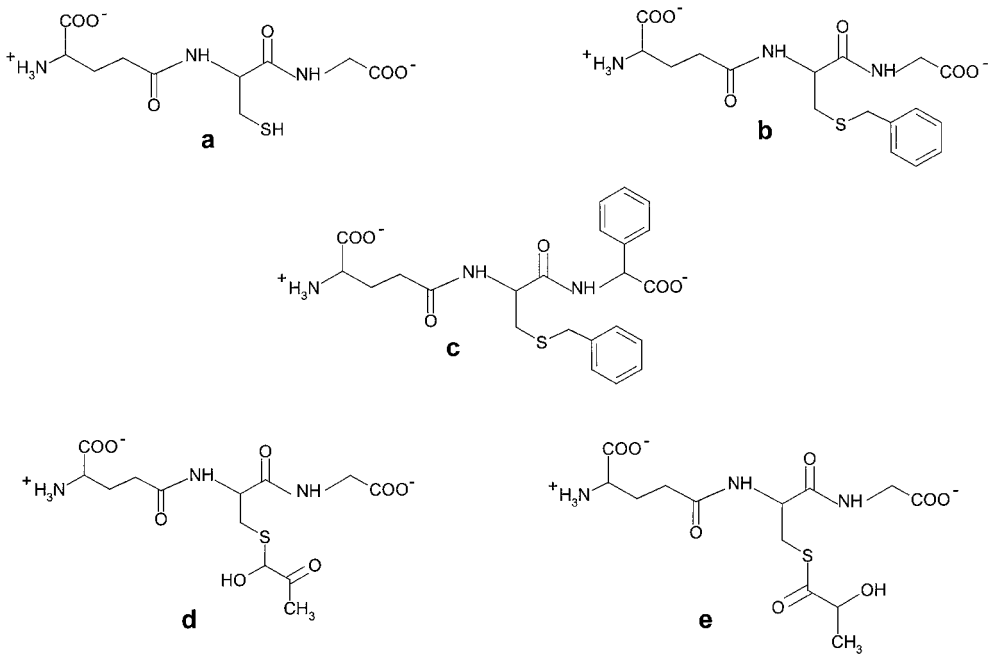

Fig. 1. Chemical structures of GSH and GSH derivatives used in the present study. a, reduced GSH. b, the inhibitor *S*-benzylglutathione. c, the inhibitor TER 117. d, the hemithioacetal formed between reduced glutathione and methylglyoxal, a glyoxalase I substrate. e, *S*-D-lactoylglutathione (a thiolester of GSH), a glyoxalase II substrate.

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glyoxalase II were obtained as detailed by Ridderström and Mannervik (1996) and Ridderström et al. (1996), respectively.

Inhibition Studies on GST P1-1 Variants. The potency of TER 117 in the inhibition of GST P1-1/Ile-105 and GST P1-1/Val-105 was determined by means of GSH competition experiments using 1 μM TER 117 and three different fixed concentrations of GSH: 0.2, 0.6, and 2.0 mM. The concentration of the second substrate, 1-chloro-2,4-dinitrobenzene (CDNB) ranged between 0.15 and 1.8 mM. In addition, the inhibitor was tested at different concentrations, 0 to 8 μM, at a CDNB concentration of 1 mM and the above GSH concentrations. Initial velocities were determined spectrophotometrically at 30°C. The conjugation reaction between GSH and CDNB was monitored at 340 nm in 1 ml of 0.1 M sodium phosphate, pH 7.0 (Habig et al. 1974).

Inhibition Studies on Glyoxalase I and II. Glyoxalase I and II activities were studied at 0.2, 0.6, and 2.0 mM GSH. The inhibition experiments were carried out in 1 ml of 0.1 M sodium phosphate, pH 7.0, at 30°C in the presence and absence of 2 μM TER 117. The formation of *S*-D-lactoylglutathione from the glyoxalase I substrate MG-SG was monitored spectrophotometrically at 240 nm according to Racker (1951). The inhibition of glyoxalase I was also studied with different TER 117 concentrations (0–5 μM) at a concentration of free GSH of 0.2 mM. In both cases, the concentration of MG-SG was varied between 20 and 400 μM. The hydrolysis of *S*-D-lactoylglutathione, catalyzed by glyoxalase II, was followed at 240 nm (Racker, 1951). The concentration of *S*-D-lactoylglutathione was varied between 10 and 800 μM. The inhibitory effect of free GSH on glyoxalase I activity was also determined from the inhibition data obtained.

Inhibition Studies Using TER 117 DEE as Inhibitor. The inhibitory effect of TER 117 DEE (100 μM) was measured under the same conditions as for TER 117. GST P1-1 was assayed at 1 mM concentrations of CDNB and GSH; glyoxalase I activity was measured with 1.6 mM MG-SG and 2 mM free GSH.

Analysis of Inhibition Data. To determine the type of inhibition, the Lineweaver-Burk equation was fitted to the inhibition data using Prism 2.0 (GraphPad, San Diego, CA). TER 117 was found to be a competitive inhibitor of both GST P1-1 and glyoxalase I, and apparent $K_I^{TER\ 117}$ values at fixed concentrations of free GSH were determined from eq. 1, describing competitive inhibition, by nonlinear regression using qnfit provided in the SIMFIT package (Bardsley et al., 1995).

$$v = \frac{V[S]}{[S] + K_m(1 + [I]/K_I)} \quad (1)$$

This equation was also used to determine K_I^{GSH} and $K_m^{MG\text{-}SG}$ values for glyoxalase I. The $K_I^{TER\ 117}$ value of glyoxalase I in the absence of GSH was determined by nonlinear regression analysis using eq. 2, in which a term for the competitive inhibitory effect of GSH has been included.

$$v = \frac{V[S]}{[S] + K_m^{MG\text{-}SG}(1 + [GSH]/K_I^{GSH} + [TER\ 117]/K_I^{TER\ 117})} \quad (2)$$

$K_m^{MG\text{-}SG}$ is the Michaelis constant for MG-SG, and K_I^{GSH} and K_I^{TER117} are the inhibition constants for GSH and TER 117, respectively.

Based on the assumption that the conjugation reaction of GSH to CDNB, catalyzed by GST P1-1, follows the equation for a rapid equilibrium random BiBi mechanism with a dead end enzyme-inhibitor complex (Segel, 1975), $K_I^{TER\ 117}$, K_m^{CDNB}, and K_m^{GSH} values were determined. This was accomplished by fitting eq. 3 to the inhibition data using qnfit in the SIMFIT package.

$$v = \frac{V[CDNB][GSH]}{K_m^{CDNB}K_m^{GSH}(1 + [TER\ 117]/K_I^{TER\ 117}) + K_m^{GSH}[CDNB] + K_m^{CDNB}[GSH] + [CDNB][GSH]} \quad (3)$$

which describes the reaction rate v as a function of three variables: the concentrations of the two substrates GSH and CDNB and of the inhibitor TER 117, competitive with both substrates. $K_I^{TER\ 117}$ is the inhibition constant, and K_m^{CDNB} and K_m^{GSH} are the Michaelis constants for CDNB and GSH, respectively.

Results and Discussion

TER 117 Inhibition of Glyoxalase System. TER 117 has until now been considered to be an inhibitor specific for GST P1-1. However, this study shows that TER 117 also affects the glyoxalase system by inhibiting glyoxalase I. This enzyme was, under physiological conditions (pH 7.0, 2 mM GSH), shown to be inhibited to almost the same extent as GST P1-1 (Table 1). An analysis of the inhibition of glyoxalase I at various concentrations of GSH and the glyoxalase I substrate MG-SG shows that TER 117 exhibits the characteristics of a linear competitive inhibitor (Fig. 2). The competitive type of inhibition (Fig. 2a) indicates that the inhibitor binds to the active site of glyoxalase I, and its linear dependence on inhibitor concentrator (Fig. 2b) implies that only one molecule of TER 117 is involved per active site.

The $K_I^{TER\ 117}$ value for glyoxalase I obtained by extrapolation of the free GSH concentration to zero is approximately 4 times higher than the $K_I^{TER\ 117}$ value for GST P1-1 (Table 2). GSH was also found to be a competitive inhibitor of glyoxalase I (data not shown). The K_I^{GSH} value was determined as 145 ± 38 μM, and the $K_m^{MG\text{-}SG}$ value was determined as 21 ± 5 μM using eq. 1. The $K_I^{TER\ 117}$ value for glyoxalase I (0.56 ± 0.05 μM) is in the same range as the K_I values previously determined for this enzyme with other GSH derivatives, such as *S*-hexylglutathione (0.37 μM), *S*-benzylglutathione (0.28 μM) (at a concentration of free GSH of 0.1 mM) (Ridderström et al., 1997), and the potent glyoxalase I inhibitor *S*-*p*-bromobenzylglutathione (K_I = 0.08 μM) (Aronsson et al., 1981). This implies that TER 117 presumably would affect the catalytic activity of glyoxalase I in cells exposed to this inhibitor. Comparison of the K_I^{GSH} value with the $K_I^{TER\ 117}$ value shows that the introduction of the benzyl group attached to the sulfur of GSH (Fig. 1a), as in *S*-benzylglutathione (Fig. 1b), and the further modification by a phenyl group on the glycine of GSH (Fig. 1c) increases the affinity of glyoxalase I for the ligand by approximately 260-fold. The only difference between TER 117 (Fig. 1c) and *S*-benzylglutathione is the phenyl group of the glycyl moiety of TER 117, which replaces a hydrogen in *S*-benzylglutathione. The crystal structure of human glyoxalase I was solved with *S*-benzylglutathione as a ligand (Cameron et al., 1997). The structure shows a lack of specific interaction with

TABLE 1

K_I values of TER 117 for GST P1-1/Val-105, GST P1-1/Ile-105, and glyoxalase I determined at various fixed concentrations of GSH

K_I values were determined at three different concentrations of GSH: 0.2, 0.6, and 2.0 mM. CDNB varied between 0.15 and 1.8 mM. MG-SG varied between 20 and 400 μM. The inhibition experiments were performed in 0.1 M sodium phosphate, pH 7.0, at 30°C. K_I value ± S.D. were determined by nonlinear regression analysis.

Enzyme	$K_I^{TER\ 117}$		
	0.2 mM GSH	0.6 mM GSH	2.0 mM GSH
		μM	
GST P1-1/Val-105	0.15 ± 0.01	0.43 ± 0.03	1.3 ± 0.1
GST P1-1/Ile-105	0.11 ± 0.01	0.34 ± 0.04	1.0 ± 0.1
Glyoxalase I	1.3 ± 0.1	2.1 ± 0.4	3.8 ± 0.6

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the glycine residue. This suggests a mobility that would make it possible to readily accommodate the phenyl group attached to the glycyl moiety of TER 117 in the active site. Such flexibility may also explain the modest difference between the values of $K_I^{S\text{-benzylglutathione}}$ and $K_I^{\text{TER 117}}$. Furthermore, the crystal structure shows that the benzyl group of *S*-benzylglutathione attached to the sulfur of the cysteinyl moiety, as in TER 117, binds in a hydrophobic cavity in the active site. These observations imply that the inhibitor TER 117 should be able to fit into the active site of glyoxalase I, which is in agreement with the present results.

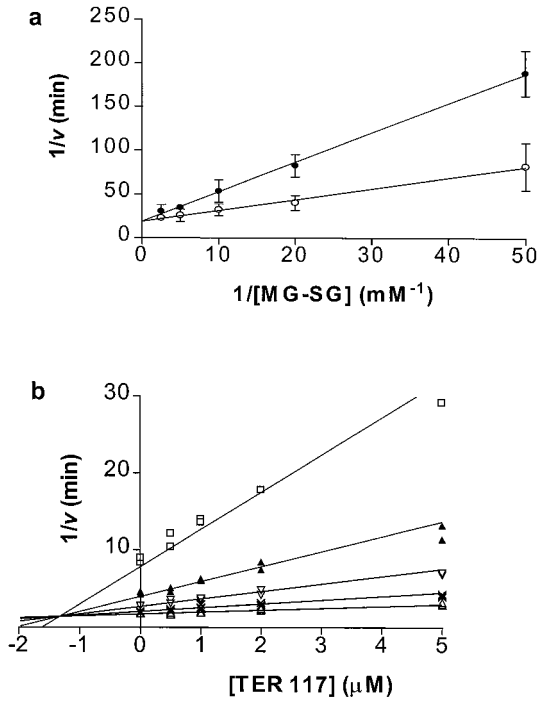

Fig. 2. Lineweaver-Burk and Dixon plots showing linear competitive inhibition of glyoxalase I by TER 117. a, TER 117 inhibition of glyoxalase I with the adduct of methylglyoxal and GSH (MG-SG) as varied substrate. ○, values corresponding to noninhibited enzyme activity. ●, values corresponding to enzyme activity in the presence of 2 μM TER 117. S.D. values are shown as error bars. b, Dixon plot. The straight lines were constructed from the parameters obtained by fitting eq. 2 to the data set using nonlinear regression analysis. Data points were obtained from measurements at 0.2 mM GSH by varying the concentration of TER 117 between 0 and 5 μM and MG-SG between 20 and 400 μM. △, 20 μM MG-SG. X, 50 μM MG-SG. ▽, 100 μM MG-SG. ▲, 200 μM MG-SG. □, 400 μM MG-SG. The negative [I] value at the intersection of the lines gives the K_I value under the experimental conditions used (0.2 mM GSH).

In contrast to glyoxalase I, glyoxalase II was not inhibited by TER 117. A concentration of TER 117 as high as 100 μM, a concentration 180-fold higher than the K_I value for glyoxalase I, did not show any detectable inhibitory effect on the glyoxalase II activity. This may appear surprising, because the substrates of the two enzymes are isomers (Fig. 1, d and e), and analogs of the substrates generally act as competitive inhibitors of both enzymes (Norton et al., 1985). However, a common feature of the most potent inhibitors of glyoxalase II is the thiolester functionality (Norton et al., 1993). This group is missing in TER 117 (Fig. 1c). The recently determined crystal structure of glyoxalase II (Cameron et al., 1999) shows that the glycine residue of GSH makes close interactions with the protein. A complementary explanation for the lack of inhibition may therefore be that the phenylglycine residue of TER 117 is too bulky for accommodation in the active site.

TER 117 Inhibition of GST P1-1. TER 117 was shown to exhibit the kinetics of a linear competitive inhibitor with respect to both CDNB and GSH (Fig. 3, a–c). The competitive effect of TER 117 indicates that the inhibitor is targeted to the active site of GST P1-1 and suggests that TER 117 would also affect activities of the enzyme with other substrates, including cytostatic drugs.

The K_I values of TER 117 determined for the two GST P1-1 variants at the various concentrations of free GSH are shown in Table 1. Under conditions that approximate the intracellular milieu (pH 7.0, 2 mM GSH), no significant difference in the inhibitory efficacy of 1 μM TER 117 could be observed between the two allelic GST P1-1 variants. The similar K_I values indicate that differences in *GSTP1* genotype would not affect the chemosensitizing effect of TER 117 in cancer patients.

Extrapolation of the K_I values to zero GSH concentration provides a good approximation of the intrinsic affinity of free enzyme for the inhibitor, normally given as the dissociation constant. The values of $K_I^{\text{TER 117}}$ for the GST P1-1 isoenzymes were not significantly different and in the submicromolar range (Table 2), demonstrating that this GSH analog binds tightly to the enzyme. The high affinity between GST P1-1 and TER 117 suggests that the inhibitor indeed is likely to act as a chemosensitizer by interfering with the GST P1-1-mediated detoxication pathway.

In the course of this study, the crystal structure of GST P1-1 in complex with TER 117 as well as with GSH was published (Oakley et al., 1997). In the complex with TER 117,

TABLE 2

Kinetic parameters of glyoxalase I, GST P1-1/Ile-105, and GST P1-1/Val-105

Measurements were carried out in 1 ml of 0.1 M sodium phosphate, pH 7.0, at 30°C. In measurements involving GST P1-1, the concentrations of CDNB and GSH ranged between 0.15 and 1.8 mM and between 0.2 and 2.0 mM, respectively. In measurements with glyoxalase I, the concentrations of MG-SG and GSH ranged between 20 and 400 μM and between 0.2 and 2.0 mM, respectively.

Enzyme	$K_I^{\text{TER 117}}$	$K_I^{\text{GSH}a}$	$K_m^{\text{MG-SG}a}$	$K_m^{\text{CDNB}b}$	$K_m^{\text{GSH}b}$
	μM	*μM*	*μM*	*mM*	*mM*
GST P1-1/Ile-105	0.14 ± 0.03[c]			0.36 ± 0.02	0.25 ± 0.01
GST P1-1/Val-105	0.12 ± 0.02[c]			0.70 ± 0.04	0.14 ± 0.01
Glyoxalase I	0.56 ± 0.05[d]	145 ± 38	21 ± 5		

[a] K_I^{GSH} and $K_m^{\text{MG-SG}}$ were determined by fitting eq. 1 to the inhibition data using nonlinear regression.
[b] K_m^{CDNB} and K_m^{GSH} were determined by nonlinear regression analysis by fitting eq. 3 to the experimental data.
[c] K_I values for GST P1-1 were determined by varying TER 117 at seven concentrations between 0 and 8 μM. The concentration of CDNB was kept constant at 1 mM and the concentration of GSH ranged between 0.2 and 2.0 mM. K_I values for GST P1-1, extrapolated to zero GSH concentration, were determined by nonlinear regression analysis by fitting eq. 3 to the experimental data.
[d] The K_I value for glyoxalase I was determined by varying TER 117 at five concentrations between 0 and 5 μM. The concentration of free GSH was kept constant at 0.2 mM and the concentration of MG-SG ranged between 20 and 400 μM. The $K_I^{\text{TER 117}}$ value for glyoxalase I extrapolated to zero GSH concentration was determined by nonlinear regression analysis using eq. 2.

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the phenyl moiety of the inhibitor interacts with the hydrophobic residues Phe-9, Val-36, and Trp-39, which together make up a hydrophobic cleft accommodating the benzyl group of the inhibitor between Phe-9 and the adjacent Tyr-109. The multiple hydrogen bonding interactions between the remainder of the GSH derivative and the residues in the GSH-binding site are the same as those in the structure with GSH as ligand. The replacement of the glycine moiety in GSH with phenylglycine in TER 117 has been shown to be responsible for the selective effect on GST P1-1 (Flatgaard et al., 1993). It affords an approximately 40-fold increase in the affinity for GST P1-1 compared with other GSTs. Comparisons with crystal structures of GSTs from other classes (Dirr et al., 1994) suggest that a phenyl group of the TER 117 glycyl moiety is too bulky to fit into the active sites and would result in sterical clashes. No part of TER 117 seems to lie in the close proximity of residue 105 in the GST P1-1 crystal structure, suggesting that any interactions between this residue and the inhibitor are very unlikely. This is in agreement with the present results showing no notable difference in the K_I values of TER 117 for the allelic GST P1-1/Ile-105 and GST P1-1/Val-105 variants.

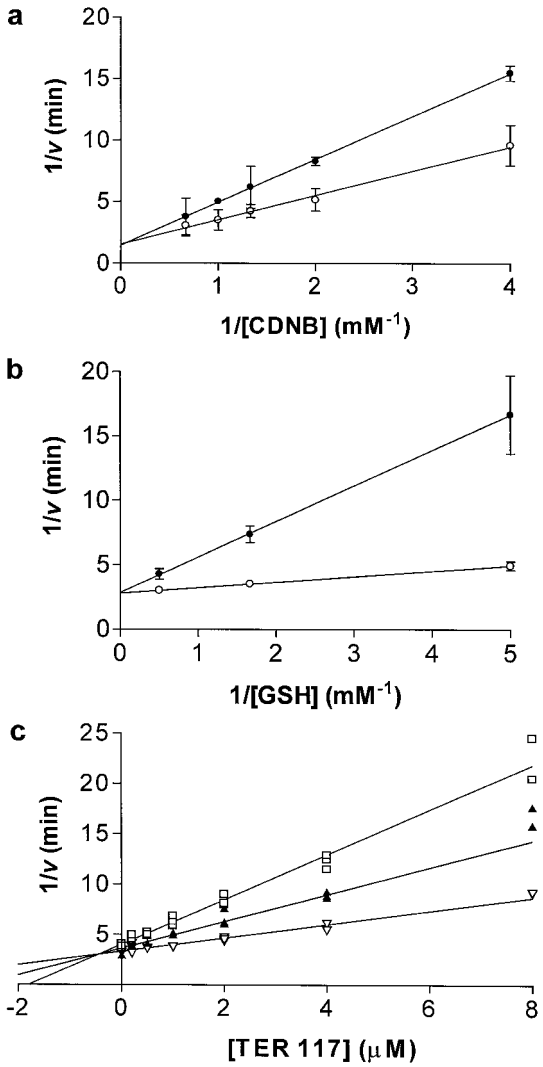

Fig. 3. Lineweaver-Burk and Dixon plots showing linear competitive inhibition of GST P1-1 by TER 117. Inhibition of GST P1-1/Val-105 with CDNB (a) and GSH (b) as varied substrate. ○, values corresponding to noninhibited enzyme activity. ●, values corresponding to enzyme activity in the presence of 1 μM TER 117. c, Dixon plot. The straight lines were constructed from the parameters obtained by fitting eq. 3 to the data set using nonlinear regression analysis. Data points were obtained from measurements at 1.0 mM CDNB by varying the concentration of TER 117 between 0 and 8 μM and GSH between 0.2 and 2.0 mM. ▽, 0.2 mM GSH; ▲, 0.6 mM GSH; □, 2.0 mM GSH.

TER 117 DEE. The diethyl ester of TER 117 exhibited no inhibitory effect on the GST P1-1 variants under the assay conditions used, nor did it inhibit glyoxalase I nor glyoxalase II. This suggests that the esterification of the two carboxyl groups prevents the establishment of charge interactions with the enzyme that are important for binding. The poor inhibition of TER 117 DEE may also be due to the bulkiness introduced by the addition of the two ethyl groups.

Potential Physiological Effects of TER 117. Experiments involving human tumor cell lines in tissue culture have demonstrated that treatment with TER 117 DEE potentiates drug sensitivity toward chlorambucil and melphalan (Morgan et al., 1996). Human GST P1-1 has been shown to be capable of catalyzing GSH conjugation to chlorambucil and thiotepa, but most of the commonly used cytostatic drugs, including melphalan, have not been shown to be good substrates for this enzyme. A suggested explanation for the potentiation is that the resistance is due to a capability of this enzyme to sequester the free drug or its toxic metabolites through nonproductive binding. Increased levels of the GST P1-1 enzyme, as occurring in the majority of the established tumor cell lines (Moscow et al., 1989, Castro et al., 1990; Tsuchida and Sato, 1992), could serve as a drug binding sink (O'Brien and Tew, 1996). TER 117 may compete in the nonproductive binding and increase the intracellular concentration of free drug. Treatment of tumor cells with TER 117 in such cases is likely to result in increased efficacy and therapeutic index of antitumor agents.

Because the glyoxalase system is involved in protection of the cells toward toxic 2-oxoaldehydes, the inhibitory effect of TER 117 on glyoxalase I may increase the intracellular concentration of 2-oxoaldehydes to toxic levels (Vince and Daluge, 1971, Papoulis et al., 1995). Human tumor cell lines often display elevated expression levels of both GST P1-1 and glyoxalase I (Thornalley, 1995). Because tumor cells have an increased flux through glycolysis, the production of the cytotoxic methylglyoxal is increased. Even though other enzymes (e.g., aldose reductase) are capable of metabolizing methylglyoxal, the metabolic pathway through the glyoxalase system is considered to be the major route (Thornalley, 1995). Thus, the inhibitory effect of TER 117 on glyoxalase I may raise the intracellular concentration of methylglyoxal and lead to a cytostatic effect or cause cell death. The administration of diesters of inhibitory GSH derivatives has indeed been found to elevate intracellular methylglyoxal concentrations and to induce apoptosis (Thornalley et al. 1996). Evidence for a role of human glyoxalase I in the cellular resistance to the cytostatic drugs adriamycin and mitomycin C has also been reported (Ranganathan et al., 1995). The potency of TER 117 is similar to those of GSH derivatives shown to elicit these effects. The inhibitory effects of TER 117 on both GST P1-1 and glyoxalase I may act in synergy and further improve the potential for successful chemotherapy.

Conclusion. The present investigation shows that TER 117 is a potent inhibitor of human glyoxalase I. Furthermore, the inhibitory effect of the inhibitor TER 117 is independent of the allelic variation in position 105 of human GST P1-1.

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The combined inhibitory effects of TER 117 on GST P1-1 and glyoxalase I may lead to raised intracellular concentrations of anticancer drugs and toxic 2-oxoaldehydes, which are otherwise inactivated by these detoxication enzymes. The use of TER 117 as an adjuvant drug in the therapy of cancer patients should therefore be able to make tumor cells more vulnerable and responsive to anticancer drugs.

Acknowledgments

We thank Telik, Inc., formerly Terrapin Technologies, Inc., for generously providing TER 117 and TER 117 DEE. Per Jemth is acknowledged for reviewing the manuscript.

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Send reprint requests to: Dr. Bengt Mannervik, Department of Biochemistry, Uppsala University, Biomedical Center, Box 576, SE-751 23 Uppsala, Sweden. E-mail: Bengt.Mannervik@biokem.uu.se

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