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INHIBITION OF GLUTATHIONE S-TRANSFERASES BY ANTIMALARIAL DRUGS POSSIBLE IMPLICATIONS FOR CIRCUMVENTING ANTICANCER DRUG RESISTANCE

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A strategy to overcome multidrug resistance in cancer cells involves treatment with a combination of the antineoplastic agent and a chemomodulator that inhibits the activity of the resistance-causing protein. The aim of our study was to investigate the effects of antimalarial drugs on human recombinant glutathione S-transferase (GSTs) activity in the context of searching for effective and clinically acceptable inhibitors of these enzymes. Human recombinant GSTs heterologously expressed in *Escherichia coli* were used for inhibition studies. GST A1-1 activity was inhibited by artemisinin with an IC_{50} of 6 μM, whilst GST M1-1 was inhibited by quinidine and its diastereoisomer quinine with IC_{50}s of 12 μM and 17 μM, respectively. GST M3-3 was inhibited by tetracycline only with an IC_{50} of 47 μM. GST P1-1 was the most susceptible enzyme to inhibition by antimalarials with IC_{50} values of 1, 2, 1, 4, and 13 μM for pyrimethamine, artemisinin, quinidine, quinine and tetracycline, respectively. The IC_{50} values obtained for artemisinin, quinine, quinidine and tetracycline are below peak plasma concentrations obtained during therapy of malaria with these drugs. It seems likely, therefore, that GSTs may be inhibited *in vivo* at doses normally used in clinical practice. Using the substrate ethacrynic acid, a diuretic drug also used as a modulator to overcome drug resistance in tumour cells, GST P1-1 activity was inhibited by tetracycline, quinine, pyrimethamine and quinidine with IC_{50} values of 18, 27, 45 and 70 μM, respectively. The ubiquitous expression of GSTs in different malignancies suggests that the addition of nontoxic reversing agents such as antimalarials could enhance the efficacy of a variety of alkylating agents.

Key words: *glutathione S-transferases; antimalarials; inhibition; drug resistance; chemomodulator*

During treatment of many cancers, there is often development of drug resistance in a tumour that was originally sensitive to treatment. This resistance compromises cancer chemotherapy and overall survival of the patients.[1,2] Frequently, resistance is also acquired to drugs not used in the treatment of that particular cancer. This phenomenon has been termed multidrug resistance (MDR) and renders cells resistant to chemotherapeutic agents in many cancer patients.[3] Among the mechanisms involved in the multidrug-resistant phenotype are alterations in drug transport resulting in impaired entry or enhanced efflux of the drug from the tumour cell.[4,5] In addition alterations in drug metabolism may result in impaired activation of pro-drugs or enhanced inactivation of the drug.[5]

The group of proteins implicated in these processes have become known as resistance-related proteins.[6–9] These include the efflux pumps, P-glycoprotein 170 (P-170) and multidrug-resistance-associated protein (MRP, glutathione-conjugate pump).[10,11] Altered target proteins include topoisomerase II and thymidylate synthetase.[5] DNA repair enzymes involved in resistance include O^6-methyl guanine-DNA methyltransferase that removes drug-induced lesions in the O^6-position of guanine.[12] The detoxifying enzymes that are involved include glutathione-dependent enzymes, glutathione transferases (GST)[13] and glutathione peroxidase.[9]

The major cytosolic isoforms of GSTs are grouped into the Alpha (A), Mu (M), Pi (P) and Theta (T) classes according to structural and catalytic properties.[14] GST Alpha is constitutively expressed in human livers, whilst GST Mu is variably expressed in human tissues.[15,16] GST P1-1 is the most abundant GST isoform in human erythrocytes[17] and is also in expressed human skin,[18] the iris of the eye[19] and human kidney.[20] There is a general association between increased GST expression and drug resistance,[21] *e.g.,* GST Alpha has been linked to drug resistance with nitrogen mustards,[22,23] GST Mu with nitrosoureas[24] and GST Pi with alkylating agents.[25] Increased GST levels have been suggested to play a role in determining the decreased sensitivity of tumour cells to alkylating agents such as chlorambucil,[26–29] acrolein[30] and ifosfamide.[31] GST P1-1 is also the most prevalent isoform expressed in tumour cell lines and neoplastic tissues[21,32,33] and may be a useful marker for clinical resistance to cytostatic drugs in different types of cancers.[34–38]

Drug resistance in cancer cells can be overcome by administration of a nontoxic reversing agent together with the anticancer agent.[39] This co-administration of an anticancer agent and a chemomodulator has been shown to increase the therapeutic effect of the drug.[1,40,41] The antimalarial drugs, quinine and mefloquine, have been used in clinical trials as potential drugs in reversing multidrug resistance in cancer patients.[8,41–44] Besides their known role as inhibitors of P-glycoprotein, these chemomodulators have been suggested to have intracellular protein targets that may be involved in drug distribution.[45] GSTs have the capacity to bind and detoxify many drugs[15] and, therefore, are possible targets for chemomodulation of drug resistance.

In cases where GSTs are thought to play a role in drug resistance, chemomodulation of therapy could involve countering the increased GST activity. This might be achieved by using inhibitors of glutathione synthesis[46] or by using GST inhibitors such as ethacrynic acid.[47] In the context of searching for effective and clinically acceptable inhibitors that may enhance the cytotoxic effects of antineoplastic agents, we have investigated the effect of antimalarials on human recombinant GST activity *in vitro.* Activity of GST was detected *in vitro* using various substrates including 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid (ETA)[48] and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU).[49] CDNB is a non-drug substrate, whilst ETA is a well-known diuretic drug that contains an αβ-unsaturated ketone moiety. BCNU is an alkylating anticancer agent used in a variety of malignant diseases, particularly in the treatment of brain tumours.[50] The denitrosation of BCNU is catalysed more efficiently by class Mu GSTs than by

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class Pi and Alpha GSTs,[24] and it has been shown that depletion of GSH imposed on GST M-overproducing cells increased toxicity of nitrogen mustards.[51]

MATERIAL AND METHODS

Most chemicals, including ethacrynic acid (ETA), were purchased from Sigma (St. Louis, MO) and BCNU (Becenum™) was obtained from Bristol-Myers Squib (Stockholm, Sweden).

Heterologous expression and purification of recombinant human glutathione transferases

Glutathione transferase A1-1, A2-2, M1b-1b, M2-2, M3-3 and P1-1, were expressed in *Escherichia coli* and prepared as described[52–55] with minor modifications. Instead of using hexylglutathione as the counter ligand to elute GST A1-1 and P1-1 from the affinity column, 50 mM glycine at pH 10 was used. This is because S-hexylglutathione binds to GSTs with high affinity and failure to remove S-hexylglutathione results in inhibition of the enzyme and does not give a true reflection of the specific activity. For GST P1-1 the procedure was as follows. *Escherichia coli* containing the plasmid pKXHP1 was grown in 50 ml of 2TYA medium for 8 hr in a culture. This culture was used for inoculation of 500 ml expression medium (10 g tryptone, 7.5 g yeast extract, 2.5 g NaCl, 5 g glycerol and 25 mg of ampicillin) in a 2 L Erlenmeyer flask, which was then placed in a rotary shaker at 37°C. At an OD_{555} of 0.2–0.3, IPTG was added to a final concentration of 0.2 mM. The culture was grown for 20 hr and the bacteria were harvested by centrifugation for 10 min at 3,000×*g* and resuspended in an equal volume of lysis buffer containing 10 mM Tris-HCl, pH 7.8, 50 mM EDTA, 15% glucose and 1 mg/ml chicken egg white lysozyme. After 1 hr incubation on ice, the cells were disrupted by sonication (Soniprobe, Dawex Instruments Ltd, UK). After the addition of phenylmethylsulfonyl fluoride to 170 μM, the soluble fraction was obtained by centrifugation at 100,000×*g* for 1 hr. For affinity chromatography purification of GSTs, the supernatant was combined with 40 ml S-hexylglutathione-Sepharose 6B affinity matrix in buffer A (10 mM Tris-HCl, pH 7.8, 1 mM EDTA, 0.2 mM dithiothreitol, 0.02% w/v NaN_3). The mixture was kept on ice with gentle periodic agitation for 1 hr. The gel was packed into a column and washed with buffer A and then buffer A fortified with 0.2 M NaCl (buffer B). GST was eluted with buffer B supplemented with 5 mM S-hexylglutathione. The eluted protein was concentrated using a PLGC membrane NMLW 10000 (Pharmacia Biotech, Uppsala, Sweden). The concentrated protein was dialysed against buffer A. The protein concentration was determined by the method of Lowry *et al.*[56]

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis and isoelectric focusing

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using purified GST fractions on 15% slab gels as described by Laemmli[57] using a Hoefer SE Mighty Small electrophoresis system or Biorad Protean electrophoresis system. Protein bands were stained with Coomassie Blue-G. Analytical isoelectric focusing was carried out on precast gels (pH 3–10) following the manufacturer's instructions (Pharmacia Biotech).

Assay of GST activity and inhibition by antimalarials

Enzyme activity was assessed by measuring the conjugating activity with CDNB, ETA[48] and denitrosation of BCNU.[49] The assay with CDNB was adapted so that absorbance was read in a SpectraMax 340 ELISA plate reader (Molecular Devices Corp., Sunnyvale, CA), whilst activity with ETA was determined using a Shimadzu UV spectrophotometer, UV1601 (Shimadzu, Tokyo, Japan). For the determination of the concentration of inhibitor at which 50% inhibition of enzyme activity was obtained (IC_{50}), the reaction mixture contained 15 μl of antimalarial in either phosphate buffer (0.1 M, 1 mM EDTA) or 95% ethanol. The ETA conjugation reaction was initiated by addition of 25 μl ETA (8 mM in ethanolic solution) and 50 μl GSH (5 mM in buffer) in a 1,000 μl mixture. The final concentration of ethanol was less than 5%. The blanks contained all components except for GST. The IC_{50} values were determined from plots of percent control activity *vs.* log inhibitor concentration (GraFit™-Erithacus Software).

Inactivation of the anticancer drug BCNU by GST M1-1

The formation of nitrite was used as an index of BCNU denitrosation. The effect of increasing the concentration of quinidine (QND) and quinine on GST M1-1 was investigated. The antimalarials were added at final concentrations of 25, 50, 75 and 100 μM. Blanks containing no enzyme but including the inhibitor were run in parallel. Conditions for assay were as described by Talcott and Levin.[58] Incubations were carried out in duplicate at 37°C in 50 ml Erlenmeyer flasks using 5 ml of 0.1 M phosphate buffer (pH 7.4), 5 mM GSH and 150–600 μg/ml GST. Samples were preincubated at 37°C for 30 sec and the substrate was added prepared from a freshly prepared stock in 99.5% ethanol solution (2 mM BCNU, final concentration). A control that did not contain GST was run in parallel. Aliquots (1 ml) were withdrawn and processed as described below for the determination of nitrite formation. In the preliminary experiment, BCNU denitrosation was found to be linear with respect to incubation time (up to 40 min) and protein concentration. Aliquots were withdrawn from each flask at 0, 10, 20, 30 and 40 min after the addition of the substrate. Each aliquot was pipetted into a 12 ml centrifuge tube containing 1 ml of chloroform. An aliquot (0.6 ml) of each aqueous phase was reextracted with 1 ml chloroform to remove traces of BCNU. After additional separation of the phases by centrifugation, 180 μl of each aqueous layer was reextracted with 1 ml chloroform to remove traces of BCNU. An aliquot (180 μl) of the aqueous phase was analysed for nitrite formation as follows. The twice-extracted aqueous aliquots were added to glass test tubes containing 5 ml 1% (w/v) sulphanilamide in 24% (v/v) HCl. Five milliliters of the coupling reagent, 0.02% (w/v) N-1-naphthylethyldiamine/ HCl in distilled water, were added to each tube with vortexing and the tubes were incubated for 20 min at 55°C. The solutions were then vortexed, the A_{540nm} measured and the nitrite concentration calculated from a nitrite standard curve. Specific activities of denitrosation were calculated from the slopes of the corresponding reaction progress curves and expressed as nmol nitrite produced/ min/mg protein. To establish if the reaction was mediated by GSTs, the effect of the inhibitor, ethacrynic acid, denitrosation was investigated at concentrations of 50, 250 and 1,250 μM. Blanks containing no enzyme but with the inhibitor were run in parallel.

Kinetic measurements

Measurement of the dependence of initial rates of reaction on CDNB concentration was done under the following conditions: 50–1,500 μM CDNB (for 12 concentrations) in the presence of 5 mM GSH in 0.1 M sodium phosphate, pH 6.5 and 1 mM Na_2EDTA at 30°C. Measurement of the dependence of initial rates of reaction on GSH concentration was carried out under similar conditions but with 50–5,000 μM GSH and 1.5 mM CDNB. Measurements were made in triplicate in microtitre plates using the SpectraMax 340 plate reader. GSTs do not strictly obey Michaelis-Menten kinetics,[59] but the maximum velocity V_{max} and the substrate concentration giving half maximal velocity K_m can be obtained by curve-fitting.[60] The kinetic data were analysed by nonlinear regression analysis using the computer program package ENZFITTER™ (Leatherbarrow). The inhibitor constant K_i was determined using secondary plots of $1/V_{max}$ *vs.* inhibitor concentration.[61]

RESULTS

Purification of heterologously expressed GSTs

Human GSTs heterologously expressed in *E. coli* were A1-1, A2-2, M1-1 (allelic variant b), M3-3 and P1-1. These were purified by affinity chromatography. The GSTs were purified to homogeneity and a single band was obtained on SDS-PAGE analyses (data

not shown). Specific activities of 84, 78, 119, 8 and 129 μmoles/min/mg protein were obtained for GSTs A1-1, A2-2, M1-1, M3-3 and P1-1, respectively. The effects of standard inhibitors on GST activity were also determined. The inhibition characteristics (IC_{50} values) of recombinant GSTs from *E. coli* were comparable with those reported previously (data not shown).[18,20]

Effects of antimalarials

The effect of antimalarial drugs on the activity of human recombinant GST activity was assessed by measuring the conjugating activity with CDNB, ETA and BCNU for GST M1-1. Figure 1 shows a plot used to determine the IC_{50} value for quinine's inhibition of GST P1-1, and Figure 2 shows the secondary plots for determination of the K_i values for the inhibitor and enzyme using V_{max} values for GSH and for CDNB. The V_{max} values were determined 3–4 times each for GSH and for CDNB. Similar graphs were plotted to determine IC_{50} and K_i values for each antimalarial using GSH together with CDNB as substrate for each of the GSTs. Results are summarised in Tables I–III. Table I shows IC_{50} values indicating potent inhibitions for artemisinin with GSTs A1-1 and P1-1, pyrimethamine with GST P1-1 and quinidine and quinine with GSTs M1-1 and P1-1. Tetracycline inhibited both GSTM3-3 and P1-1 but with higher IC_{50} values. Chloroquine, primaquine and sulphadoxine did not inhibit GSTs at the concentrations that were used.

The effects of the different antimalarials on the kinetic parameters $V_{max}^{GSH/CDNB}$ and $K_m^{GSH/CDNB}$ of GSTs were studied. Table II shows typical results for the effects of quinine on GST P1-1 kinetic parameters. The K_i or K'_i values determined with CDNB was generally within the same range as the K_i or K'_i determined with GSH for each particular drug (Table III). Similarly, the IC_{50} value obtained for each drug with CDNB as substrate was generally in the same range as the K_i or K'_i value for the drug (Table III). The IC_{50} and K_i values obtained (except for pyrimethamine) are below expected peak plasma concentrations of the drugs during therapy of malaria (Table III).

Using ETA as a substrate, GST P1-1 activity was inhibited by pyrimethamine, quinidine, quinine and tetracycline with IC_{50} values of 18–70 μM (Table IV). Results obtained with CDNB as substrate are shown in the table for comparison. Artemisinin did not inhibit GST P1-1 activity towards ETA at the concentrations that were used in contrast to what was observed with CDNB as substrate, and, except for tetracycline, IC_{50} values were higher with ETA as substrate.

Effects of antimalarials on the denitrosation of BCNU by GST M1-1

The data using recombinant human GSTs has shown that quinidine and quinine are effective inhibitors of GSTM1-1 and P1-1 activity with CDNB. Because of the role of GST M1-1 in the denitrosation of BCNU, further work was performed to evaluate the effect of these cinchona alkaloids on the denitrosation of BCNU *in vitro*. Spontaneous nitrite formation was subtracted from experimental values and was equivalent to 0.11 ± 0.02 nmol/min. Quinidine was shown to reduce the activity of GST M1-1 in a concentration-dependent manner with an IC_{50} of 112 μM on extrapolation of the data on Figure 3. Quinidine, thus, was found to have an inhibitory effect toward GST M1-1, monitored both by GSH-dependent CDNB metabolism and GSH-dependent denitrosation of BCNU.

DISCUSSION

Our study has shown that some antimalarials are potent inhibitors of the conjugation of CDNB by human recombinant GSTs

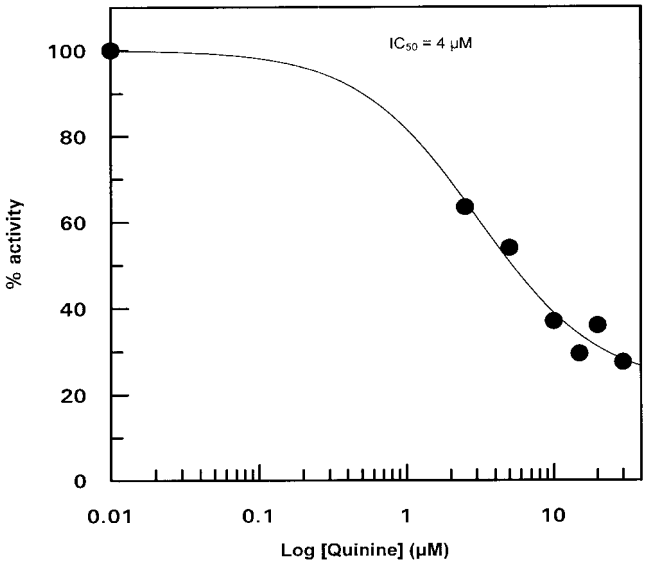

FIGURE 1 – Inhibition of GST P1-1 by quinine using CDNB as the substrate. The IC_{50} value is inhibitor concentration giving 50% inhibition of the enzyme activity in the standard assay system with 1 mM 1-chloro-2,4-dinitrobenzene and is obtained from plots of percent activity *vs.* log inhibitor concentration (GraFit™-Erithacus Software).

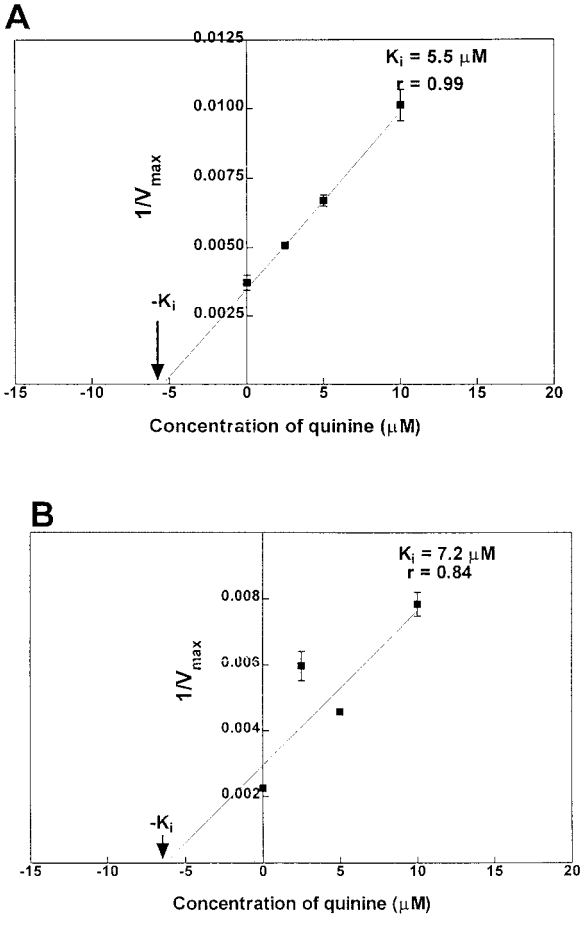

FIGURE 2 – Secondary plot of $1/V_{max}$ *vs.* antimalarial concentration to determine the K_i for quinine and GST P1-1 with respect to GSH(A) and CDNB (B). Each point represents the average of 3 values for $1/V_{max}$ with CDNB and GSH as substrates as described in Material and Methods. r represents the correlation coefficient when the data were subjected to a linear regression analysis. V_{max} values were determined for the 2 substrates GSH and CDNB from kinetic data that were analysed as described in the Material and Methods.

TABLE I – IC_{50} VALUES FOR HUMAN RECOMBINANT GLUTATHIONE S-TRANSFERASES WITH SELECTED ANTIMALARIALS IC_{50} (μM)[1]

Antimalarial	GST isoform				
	A1-1	A2-2	M1-1	M3-3	P1-1
Artemisinin	6	NI[3]	NI	NI	2
Chloroquine	60% at 100 μM[2]	NI	57% at 100 μM	NI	NI
Primaquine	60% at 100 μM	NI	NI	NI	NI
Pyrimethamine	60% at 20 μM	NI	NI	NI	1
Quinidine	70% at 2 μM	NI	12	60% at 50 μM	1
Quinine	NI	NI	17	NI	4
Sulphadoxine	NI	NI	NI	60% at 50 μM	NI
Tetracycline	70% at 100 μM	NI	NI	47	13

[1]The IC_{50} values are inhibitor concentrations giving 50% inhibition of the enzyme activity in the standard assay system with 1 mM 1-chloro-2,4-dinitrobenzene (CDNB).–[2]The figures with percentages indicate the residual activity at that concentration, since in these cases, an increase in the antimalarial concentration did not cause a decrease to below 50% original activity. The figures represent the mean of 4 determinations.–[3]NI indicates that there was no inhibition with the particular inhibitor at 100 μM concentration.

TABLE II – THE EFFECTS OF QUININE ON KINETIC PROPERTIES OF GST P1-1 WITH 1-CHLORO-2,4-DINITROBENZENE AS ELECTROPHILIC SUBSTRATE

Concentration of QN (μM)	K_{cat}^{GSH} (S^{-1})	K_{cat}^{CDNB} (S^{-1})	K_m^{GSH} (mM)	K_m^{CDNB} (mM)	K_{cat}/K_m^{GSH} (S^{-1} mM^{-1})	K_{cat}/K_m^{CDNB} (S^{-1} mM^{-1})
0	92.56 ± 4.09	119.06 ± 2.60	0.321 ± 0.05	0.645 ± 0.03	290.47 ± 29	184.5 ± 3.66
2.5	63.74 ± 0.20	59.80 ± 7.00	0.263 ± 0.02	0.165 ± 0.03	242.56 ± 13.27	365.90 ± 37
5	59.28 ± 2.34	66.82 ± 3.91	0.247 ± 0.02	0.549 ± 0.024	244.61 ± 17.83	121.80 ± 5.18
10	36.62 ± 2.7	41.73 ± 1.56	0.164 ± 0.03	0.193 ± 0.03	225.33 ± 19.24	219.01 ± 26

TABLE III – K_I AND IC_{50} VALUES FOR INHIBITION OF THE CONJUGATION OF 1-CHLORO-2,4 DINITROBENZENE (CDNB) TO GLUTATHIONE BY *E. COLI*-EXPRESSED HUMAN RECOMBINANT GLUTATHIONE S-TRANSFERASES[1]

Isoform/compound	K_i^{GSH} (K'_i)	K_i^{CDNB} (K'_i) (μM)[2]	IC_{50}	Type of inhibition		Peak plasma concentration (μM)
				GSH	CDNB	
GST M1b-1b						
Quinidine	4.0	4.3	12	Uncomp	Uncomp[3]	30
Quinine	5.0	8.5	17	Uncomp	Part. Uncomp[4]	30
GST A1-1						
Artemisinin	38 (57)	8.0 (75)	6	Mixed	Mixed	10
GST M3-3						
Tetracycline	36.0	41.0	47	Mixed	Mixed	12–45
GST P1-1						
Artemisinin	2.4 (17)	33 (17)	2	Mixed	Mixed	10
Pyrimethamine	0.9	1.7	1	Uncomp	Uncomp	0.6
Quinine	5.5	7.2	4	Uncomp	Uncomp	30
Quinidine	6.0	9.2	1	Uncomp	Uncomp	30
Tetracycline	0.4 (17)	4.0 (18)	13	Uncomp	Uncomp	12–45

[1]Values for plasma antimalarial concentrations obtained during treatment with the drugs were obtained from Berqgvist and Churchill,[65] Edwards[66,67] and Goodman and Gillman.[68]–[2]The K_i (K'_i) values were determined for triplicate $1/V_{max}$ values for each [I]. The IC_{50} was determined in quadruplicate for each inhibitor.–[3]Uncomp, uncompetitive inhibition.–[4]Part. Uncomp, partial uncompetitive inhibition.

with IC_{50} values from 1–46 μM and K_i values generally below 20 μM. Of the 8 antimalarials noted to affect activity when using CDNB as a substrate, pyrimethamine and quinine were the most potent inhibitors. GST P1-1 was the enzyme most susceptible to inhibition. The K_i for artemisinin using CDNB for GST A1-1 was lower than the plasma concentration of the drug so that this drug would bind to isoform with a higher affinity to the H-site (K_i = 8 μM) compared to the G-site (K_i = 38 μM).

Considering the IC_{50} or K_i values relative to the expected plasma concentration of the drugs in the body during therapy, our results with CDNB as a substrate show that some antimalarials may be effective inhibitors of GSTs *in vivo*. Quinine and Quinidine as inhibitors for GSTs M1-1 and P1-1, artemisinin for GSTs A1-1 and P1-1 and tetracycline for GST M3-3 may be worthy of future investigations in cell lines with a view to their possible clinical use in chemomodulation.

Chemomodulation during chemotherapy could involve targeting GSTs responsible for the breakdown of several alkylating agents. Quinine has already been demonstrated to reverse resistance to doxorubicin in leukaemia cell lines[45] and as a chemomodulator in breast cancer patients who failed to respond to combination therapy of cyclophosphamide, vincristine, adriamycin and dexameth-

TABLE IV – K_M AND IC_{50} VALUES FOR ANTIMALARIALS FOR HUMAN RECOMBINANT GST P1-1 ACTIVITY TOWARDS CDNB AND ETA[1]

Parameter	CDNB	ETA
	(μM)[2]	
K_m	1,200	29
IC_{50}		
Artemisinin	2	>100
Quinidine	1	70
Quinine	4	27
Tetracycline	13	18
Pyrimethamine	1	45

[1]K_m values for CDNB and ETA for GST P1-1 were obtained from Johansson *et al.*[69]–[2]The IC_{50} value is inhibitor concentration giving 50% inhibition of the enzyme activity in the standard assay systems with 1 mM 1-chloro-2,4-dinitrobenzene and ethacrynic acid was obtained from plots of percent activity *vs.* log inhibitor concentration. Activities were determined with CDNB in quadruplicate in a Spectra-Max 340 ELISA reader equipped with the kinetics mode and with ETA in a Shimadzu UV 1601 spectrophotometer.

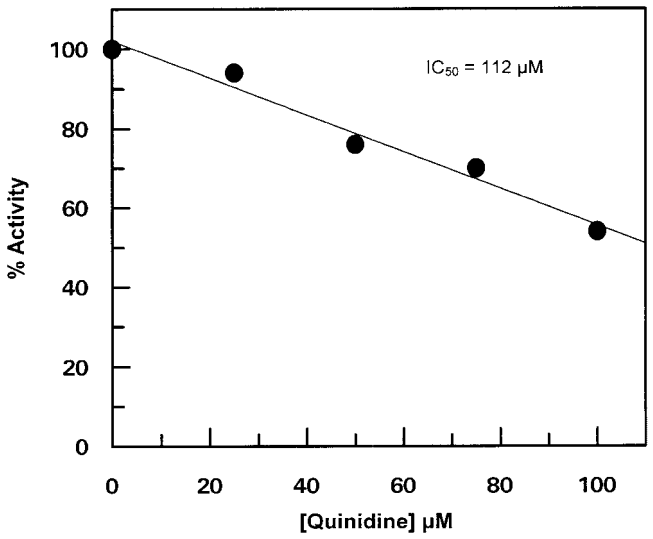

FIGURE 3 – Effects of quinidine on the denitrosation of BCNU by GST M1-1.

asone (CAVD).[40] Quinine as a nontoxic chemomodulator thus enhanced the clinical activity of anticancer drugs in combination therapy.

The mechanism by which quinine is thought to reverse drug resistance in MDR is by inhibiting the function of the P-glycoprotein by serving as a competing substrate or by acting as an allosteric modulator of the protein.[62,63] The results obtained in our study, showing that quinine is an effective inhibitor of GST P1-1 *in vitro*, suggest that inhibition of GST P1-1 *in vivo* may be one of the mechanisms to explain the effects of quinine as a chemomodulator in clinical studies. Studies have demonstrated that there is a tendency for simultaneous expression of P-170 and GST P1-1 in some cancer types.[6,25] Since resistance in cancer treatment is multifactorial, the use of a chemomodulator with more than one target is likely to be of great therapeutic advantage.

Glutathione transferases have been suggested to be necessary for the efflux of anticancer drugs from tumour cells.[64] Resistance to chlorambucil in MCF7 breast carcinoma cells showed that GSTs (A1, P1) and MRP1 acted in synergy to protect cells from the cytotoxicity of chlorambucil and ethacrynic acid, respectively.[23,28] These proteins may present the hydrophobic ligands to efflux pumps either conjugated to GSH or nonconjugated.[11]

Although most studies of GST P1-1 activity in normal and tumour samples use the electrophilic substrate CDNB, this is a nondrug substrate and the question remains as to the suitability of this substrate for extrapolation to other substrates. Whilst artemisinin inhibited GST activity with CDNB as a substrate, inhibition was not observed with ETA as a substrate. ETA is the substrate that is conventionally used to discriminate GST P1-1 from other isoforms. It is interesting to note that with ETA as substrate, the IC_{50} values for all the antimalarials, except tetracycline, were considerably higher than those obtained with CDNB as substrate. In this respect, it was also noted that although quinidine was an effective inhibitor of GST M1-1 using CDNB, the IC_{50} value was greater than 100 μM when using BCNU as a substrate. Extrapolation of results from inhibition studies with CDNB to other substrates may not be valid as exemplified well here by artemisinin, which inhibited CDNB conjugation but not ETA conjugation. The most appropriate substrate for GSTs for inhibition studies would be the anticancer drug that is a substrate of the enzyme. Most investigators routinely use only CDNB as the substrate.

Further work is needed to determine whether antimalarials inhibit other resistance-related proteins like glutathione peroxidase, topoisomerase II and DNA repair enzymes.

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