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Inhibition of Glutathione Reductase by Plant Polyphenols

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ABSTRACT. The effects of forty-one plant polyphenols on the activity of glutathione reductase (GSH-RD) were studied. These polyphenols showed varying degrees of concentration-dependent inhibition on the enzyme, with IC_{50} values that varied from approximately 40 μM to 1 mM. 4′-Hydroxychalcone and tannic acid were among the more potent inhibitors, with IC_{50} values of 47.3 and 50.4 μM, respectively. Different classes of polyphenols varied in potency in the following order: chalcones > tannic acid > flavonoids > coumarins > catechins. Analysis of structure–activity relationships showed certain chemical structures to be important for the inhibition of GSH-RD: (a) C-5 and C-7 hydroxylations in the A-ring, a carbonyl group at C-4, and the B-ring attached to C-2 in flavonoids; (b) C-2′ and C-4′ hydroxylations in chalcones; and (c) C-6 and C-7 hydroxylations in coumarins. The inhibition of GSH-RD by tannic acid and quercetin was time dependent and irreversible, whereas that by 4′-hydroxychalcone and esculin was reversible but not time dependent. Enhanced inhibition of GSH-RD by the four polyphenols 4′-hydroxychalcone, quercetin, butein, and acacetin was observed in the presence of NADPH. Kinetic studies showed that both tannic acid and 4′-hydroxychalcone exhibited non-competitive inhibition on GSH-RD towards glutathione disulfide. BIOCHEM PHARMACOL 54;9: 1047–1053, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. glutathione reductase; flavonoids; chalcones; polyphenols; inhibition

GSH[‖] and GSH-dependent enzymes participate in the detoxification of endobiotics and xenobiotics including many anticancer alkylating agents [1, 2]. High levels of GSH and overexpression of GSTs have been reported to confer chemo-resistance to tumor cells against alkylating agents [3, 4]. Depletion of GSH by buthionine sulfoximine and inhibition of GSTs by ethacrynic acid could sensitize the drug-resistant cells to these drugs [5, 6]. GSH-RD, which catalyzes the reduction of oxidized-GSH to the reduced form, is important in maintaining the high levels of reduced GSH in cells [7, 8]. The latter is essential for GSH conjugation of many anticancer drugs [9]. GSH-RD also has been shown to be overexpressed in human tumor tissues and in mouse tumor cell lines, and a high activity of the enzyme may be associated with tumor growth and resistance mechanisms against anticancer drugs [10]. Some flavonoids have been shown to inhibit GSH-RD [11]. Interestingly, we found that flavonoids and other polyphenols could sensitize human colon adenocarcinoma cells to chlorambucil at low and non-toxic concentrations [12]. The inhibitory effects of these plant polyphenols on rat liver GSTs and on the transport of GSH conjugates by human colon tumor cells were also demonstrated in our laboratory [13, 14], and this may contribute to their ability to sensitize human tumor cells to chlorambucil. However, these effects alone were not of a sufficient magnitude to account for their sensitizing actions on human colon adenocarcinoma cells to chlorambucil. The inhibition of GSH-RD may also be involved. This study investigated the inhibitory effects of a variety of polyphenols on GSH-RD.

MATERIALS AND METHODS

Chemicals

Flavonoids (see Table 1) and other polyphenols (see Table 2) were obtained from Extrasynthese (Genay, France). GSSG, NADPH, and GSH-RD (Type VII, from bovine intestinal mucosa) were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Determination of GSH-RD Activity

GSH-RD activity was assayed according to the method of Racker [15]. The reaction mixture consisted of 50 mM potassium phosphate buffer containing 1 mM EDTA, pH 7.4, with final concentrations of 1 mM GSSG and 0.2 mM NADPH. The reaction was started by the addition of NADPH. Consumption of NADPH was monitored at 37° for 3 min on a spectrophotometer at 340 nm. Enzyme

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‖ *Abbreviations:* GSH, glutathione; GSH-RD, glutathione reductase; GSTs, glutathione S-transferases; and GSSG, glutathione disulfide.

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activity was expressed as nanomoles of NADPH oxidized per minute per milligram of protein at 37°, using an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

Inhibition Studies

Studies of the inhibition of GSH-RD by polyphenols were carried out at 37° and pH 7.4, using GSSG as a substrate. The 10 mM stock solutions of polyphenols were prepared freshly in absolute alcohol or DMSO. Then aliquots of these solutions were added to the reaction mixture to yield a final concentration ranging from 10 μM to 1 mM. In all assays, the final concentration of ethanol or DMSO did not exceed 2% (v/v), and this concentration of ethanol or DMSO did not affect GSH-RD activity. The IC_{50} values were calculated by linear regression of no less than five points in the range of 20 to 80% inhibition. Each point represents the mean of at least two determinations.

Time-Dependent Inhibition of GSH-RD by Polyphenols

In these experiments, the concentrations of tannic acid, 4′-hydroxychalcone, quercetin, and esculin were held constant at 30, 30, 60, and 120 μM, respectively, and then GSH-RD was incubated with these inhibitors for various periods: 10 min, 30 min, 1 hr, 2 hr, and 4 hr. Then GSH-RD activity was determined as described above.

Effects of NADPH on the Inhibition of GSH-RD

These studies were carried out by preincubation of GSH-RD with tannic acid (50 μM), 4′-hydroxychalcone (50 μM), quercetin (100 μM), butein (100 μM), and acacetin (100 μM) in the absence or presence of NADPH. The activities of GSH-RD in the absence or presence of NADPH were determined and compared using Student's *t*-test [16].

Reversibility of Inhibition

Dialyzing experiments were carried out using standard cellulose dialysis tubing (Spectrum Medical Industries, Los Angeles, CA, U.S.A.), having cut-off of molecular weight in the range of 12,000–14,000. The GSH-RD (0.45 μg protein/mL) was incubated in aliquots of dialyzing buffer (4 mL) containing one of the following compounds: tannic acid (80 μM), 4′-hydroxychalcone (80 μM), quercetin (100 μM), and esculin (400 μM) at 25° for 20 min. Aliquots (500 μL) of each incubation mixture were removed, and GSH-RD activity of the incubation mixture was measured. Additional aliquots of the remaining incubation mixture (3.5 mL) were dialyzed against the dialyzing buffer which consisted of 50 mM potassium phosphate and 1 mM EDTA, pH 7.4, at 4° for 24 hr. GSH-RD activities were also determined after dialysis. The controls were without inhibitors and were treated in the same way as described for the test samples.

Kinetic Studies

Kinetics of the inhibition of GSH-RD activity by tannic acid and 4′-hydroxychalcone were studied using various concentrations (0.05 to 1 mM) of GSSG in the presence of 40 or 80 μM tannic acid and 4′-hydroxychalcone, respectively.

RESULTS

Effects of Polyphenols on GSH-RD Activity

Forty-one polyphenols were tested for their effects on GSH-RD activity. Their potencies differed widely in inhibiting GSH-RD activity; IC_{50} values calculated from linear regression for flavonoids and other polyphenols are listed in Tables 1 and 2, respectively. Concentration-dependent inhibition was observed for most of the polyphenols tested. In the range of 20 to 80% inhibition, the concentration–response curves were found to be linear, with correlation coefficient values between 0.92 and 0.99.

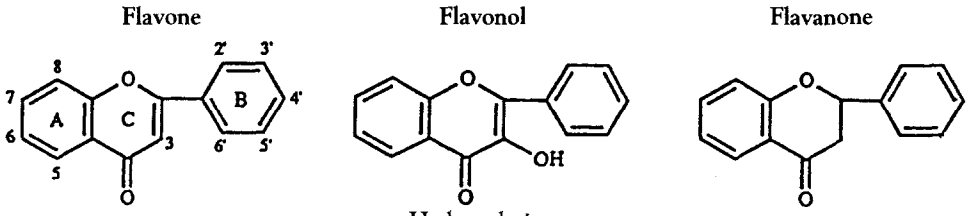
Reversibility of the Inhibition of GSH-RD by Polyphenols

GSH-RD was incubated with polyphenols at 25° for 20 min. The activity of GSH-RD was inhibited up to 79, 85, 57, and 76% by 80 μM 4′-hydroxychalcone, 80 μM tannic acid, 100 μM quercetin and 400 μM esculin, respectively (Fig. 1). The inhibitors were then removed by dialyzing the reaction mixtures. The inhibition of GSH-RD by 4′-hydroxychalcone and esculin could be reversed significantly after dialysis for 24 hr ($P < 0.0001$), but the activities still could not be restored to the control level. The inhibition by tannic acid and quercetin was irreversible. Dialysis of the reaction incubates for 24 hr was unable to restore the inhibited GSH-RD activity. In fact, the inhibition was enhanced significantly after 24 hr dialysis ($P < 0.0001$, Fig. 1).

Time-Dependent Inhibition of GSH-RD by Plant Polyphenols

Time–course experiments were also conducted with 4′-hydroxychalcone, tannic acid, quercetin, and esculin. In all cases, GSH-RD activity fell rapidly over the first 10 min. At 10 min, tannic acid was the most potent inhibitor. However, after 10 min there were differences in the inhibitory behavior of the four compounds. For tannic acid and quercetin, their inhibitory effects increased gradually over 4 hr. At 4 hr, the inhibition of GSH-RD by 30 μM tannic acid was 97% as compared with 21% at 10 min. The time-dependent inhibition of GSH-RD by quercetin showed a pattern similar to that of tannic acid. Fifteen percent and sixty-two percent inhibition were observed at 10 min and 4 hr, respectively. In contrast, the inhibition of GSH-RD by 4′-hydroxychalcone and esculin hardly changed over the period from 10 min to 4 hr (Fig. 2).

TABLE 1. Inhibition of GSH-RD by flavonoids*



Name	Class	Hydroxylation pattern	Substitution	IC_{50} (μM)
Acacetin	Flavone	5,7		63.5
Naringenin	Flavanone	5,7,4′		65.6
Quercitrin	Flavonol	5,7,3′,4′	C-3 = −rhamnose	69.4
Fustin	Flavanone	3,7,3′,4′		72.2
Quercetin	Flavonol	3,5,7,3′,4′		73.2
Apigenin	Flavone	5,7,4′		75.2
Isoquercitrin	Flavonol	5,7,3′,4′	C-3 = −glucose	84.5
Diosmin	Flavone	5,3′	C-7 = −rutinose C-4′ = $-OCH_3$	89.4
Luteolin	Flavone	3,5	C-4′ and C-7 = $-OCH_3$	94.0
Myricetin	Flavonol	3,5,7,3′,4′,5′		96.8
Isorhamnetin	Flavone	3,5,7,4′	C-3′ = $-OCH_3$	104.5
Daidzein	Isoflavone	7,4′	B-ring at C-3	113.5
Genistein	Isoflavone	5,7,4′	B-ring at C-3	116.3
Morin	Flavonol	3,5,7,2′,4′		118.7
Myricitrin	Flavonol	3,5,7,4′,5′	C-3 = rhamnose	124.7
Kaempferol	Flavonol	5,7,4′		126.5
Apiin	Flavone	5,4′	C-4 = −O − glucose − api†	128.3
Fisetin	Flavone	3,7,3′,4′		129.7
Galangin	Flavone	3,5,7		180.4
7-Hydroxyflavanone	Flavanone	7		268.2
Biochanin A	Isoflavone	5,7	B-ring at C-3	276.2
Diosmetin	Flavone	5,7,3′	C-4′ = $-OCH_3$	279.7
Naringen	Flavanone	7,4′	C-4 = $-OCH_3$ C-5 = rhamnose − glu†	313.8
4′-Hydroxyflavanone	Flavanone	4′		580.7
Flavanone	Flavanone			869.1
(+)Catechin‡	Flavan-3-ol	3,5,7,3′,4′		>1000
(−)Epicatechin‡	Flavan-3-ol	3,5,7,3′,4′		>1000

* The flavanoid concentrations used were in the range of 10 μM to 1 mM. GSH-RD activity was measured according to the method of Racker [15]. The IC_{50} values were calculated by linear regression of no less than five points in the range of 20–80% inhibition.

† -glu, -glucose; -api, -apiose.

‡ Flavonoids without a carbonyl group at C-4 and a double bond between C-2 and C-3.

Effects of NADPH on the Inhibition of GSH-RD by Polyphenols

GSH-RD was incubated with five polyphenols in the presence or absence of NADPH. As shown in Table 3, except for tannic acid, preincubation with NADPH (a cofactor required for GSH-RD activity) enhanced significantly ($P < 0.01$) the inhibition by all the compounds tested.

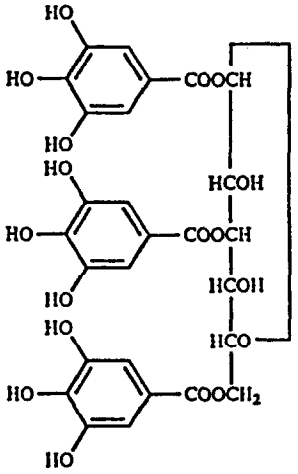
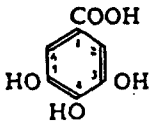
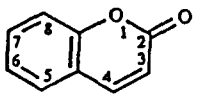
Inhibitory Kinetic Studies of GSH-RD

4′-Hydroxychalcone and tannic acid were selected for the kinetic experiments. From the Lineweaver–Burk plot analysis, both tannic acid and 4′-hydroxychalcone showed non-competitive inhibition towards GSSG (V_{max} decreased, while K_m remained unchanged) (Fig. 3).

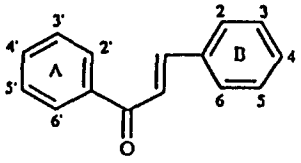
DISCUSSION

An analysis of the structure–activity relationship indicated that various classes of polyphenols exhibited the following order of potency for the inhibition of GSH-RD: chalcones > tannic acid > flavonoids > coumarins > catechins. A similar potency order also was observed for the inhibition of GSTs by polyphenols [13]. In the present study, for chalcones, the hydroxylation substitution at the C-4′ position was obviously associated with their inhibitory potencies on GSH-RD (4′-hydroxychalcone, butein, phloretin, and phloredzin with hydroxylation at C-4′ are more potent inhibitors than chalcone and 2′-hydroxychalcone). However, in our previous report, for the inhibition of rat liver GSTs by chalcones, hydroxylation substitutions at C-2 and C-3 were more important [13]. Generally, as in the inhibition of other enzymes by plant polyphenols [13,

TABLE 2. Inhibition of GSH-RD by other polyphenols

Compounds	Structure	IC_{50} (μM)
Tannic acid		50.4
Gallic acid		818.6
Coumarin		>1000

	C-2	C-6	C-7	C-8	
Daphnetin	H	H	OH	OH	234.5
Esculin	H	OH	OH	H	288.2
2-Hydroxycoumarin	OH	H	H	H	>1000

Chalcone  97.0

	C-2	C-3	C-4	C-2′	C-3′	C-4′	C-6′	
4′-Hydroxychalcone	H	H	H	H	H	OH	H	47.3
Phloredzin	H	H	OH	O-glu	H	OH	OH	68.1
Butein	H	OH	OH	H	H	OH	OH	70.8
Phloretin	H	H	OH	OH	H	OH	OH	71.5
2′-Hydroxychalcone	H	H	H	OH	H	H	H	82.0
2-Hydroxychalcone	OH	H	H	H	H	H	H	123.2
Marein	H	OH	OH	OH	OH	OH	H	176.1

The polyphenol concentrations used were in the range of 10 μM to 1 mM. The GSH-RD activity was measured according to the method of Racker [15]. The IC_{50} values were calculated by linear regression of no less than five points in the range of 20–80% inhibition.

17–19], hydroxylation of the polyphenols is also an important structural feature for the inhibition of GSH-RD. For instance, tannic acid with polyhydroxylations is a potent inhibitor. The inhibitory effects of naringenin (5,7,4′-trihydroxyflavanone), 7-hydroxyflavanone, and flavanone are noted to be in a decreasing order of potency. In addition, when compared with coumarin, the inhibition by daphnetin and esculin (both of which are dihydroxycoumarins) was enhanced significantly (Tables 1 and 2). By comparing the potencies of flavanoids that belong to different classes but possess identical hydroxylation patterns, the following order of potency was observed: flavanone (naringenin) > flavone (apigenin) > flavonol (kaempferol). Interestingly, when the B-ring of the flavonoid molecule is attached to the C-3 position (i.e. isoflavone class), their inhibitory potencies decreased (e.g. daidzein, genistein, and biochanin A). Coumarins (without B-ring) also exhibited reduced inhibitory potency. In sum-

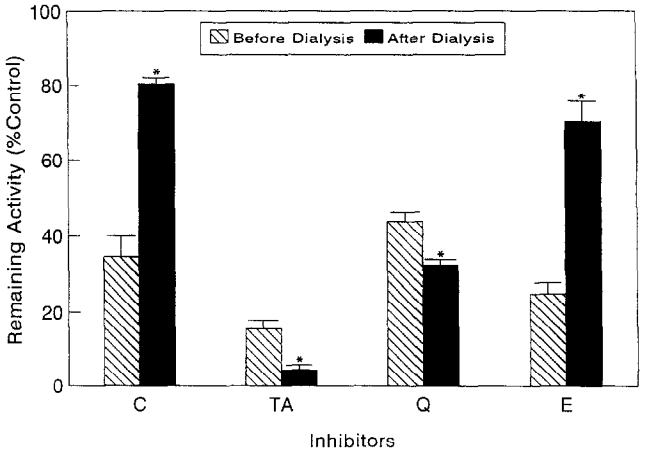


FIG. 1. Effects of dialysis on the inhibition of GSH-RD by plant polyphenols. Experiments were carried out as described in "Materials and Methods." GSH-RD activities after dialysis were significantly different from those before dialysis (*$P < 0.0001$). Control activities before and after dialysis were 149.7 and 141.9 nmol/min/mg protein, respectively. Values are means ± SD of triplicate experiments. Abbreviations: C, 4′-hydroxychalcone; TA, tannic acid; Q, quercetin; and E, esculin.

mary, C-5 and C-7 hydroxylations in the A-ring, a carbonyl group at C-4, and the B-ring attached to C-2 in flavanoids; C-2′ and C-4′ hydroxylations in chalcones; and C-6 and C-7 hydroxylations in coumarin are important chemical features for the inhibition of GSH-RD. Catechins and gallic acid are poor inhibitors, possibly due to the absence of some of the above-mentioned chemical structural features that are required for inhibitory potency on GSH-RD.

Dialysis experiments showed that the inhibition of GSH-RD by 4′-hydroxychalcone and esculin was reversible, but the activity of GSH-RD after dialysis still did not return to the control level. This finding indicates that although the inhibition by these two compounds is reversible, the attachment of the inhibitors to the enzyme requires a treatment more drastic than dialysis for total recovery of the enzyme activity. However, inhibition of GSH-RD by tannic acid and quercetin was irreversible. These results are in accord with those obtained from time–course studies of the inhibition of GSH-RD. Irreversibility of inhibition by tannic acid and quercetin may be explained by their time-dependent inhibition (enhanced inhibition at longer exposure time to the two compounds), while the inhibition by 4′-hydroxychalcone and esculin was reversible but not time dependent. This suggested different mechanisms for the inhibition of GSH-RD by the different classes of polyphenols.

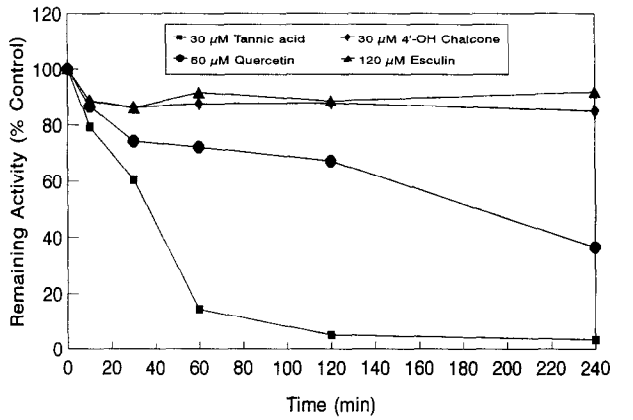


FIG. 2. Time-dependent inhibition of GSH-RD by plant polyphenols. GSH-RD was incubated with plant polyphenols for the indicated periods. GSH-RD activity was determined by the method described in "Materials and Methods." GSH-RD activities of controls at 10 min, 30 min, 1 hr, 2 hr, and 4 hr were: 149.1, 147.6, 133.8, 128.2, and 119.4 nmol/min/mg protein, respectively. Data points are means of three independent experiments.

TABLE 3. Effects of NADPH on the inhibition of GSH-RD by polyphenols

Polyphenols	Remaining activity (% of control) + NADPH	− NADPH
4′-Hydroxychalcone, 50 μM	51.2 ± 1.86	76.9 ± 8.80
Tannic acid, 50 μM	11.7 ± 3.51	13.8 ± 1.12
Quercetin, 100 μM	24.7 ± 1.83	43.7 ± 2.99
Butein, 100 μM	18.7 ± 2.03	32.6 ± 3.24
Acacetin, 100 μM	52.8 ± 2.81	67.0 ± 3.45

Experiments were performed by incubation of GSH-RD with polyphenols in the presence or absence of 100 μM NADPH for 60 min at 25°. Then GSH-RD activity was measured as described in "Materials and Methods." Except for the incubate with tannic acid, GSH-RD activities of the other incubates in the presence of 100 μM NADPH were significantly different from those without NADPH ($P < 0.01$, Student's t-test). Control GSH-RD activities in the presence and absence of NADPH were 130.2 and 151.1 nmol/min/mg protein, respectively. Values are means ± SD of three independent experiments.

The inhibition of GSH-RD by the polyphenols (except for tannic acid) was enhanced in the presence of NADPH. This suggested that inhibition of GSH-RD by these compounds is dependent on the redox state of the enzyme. In the reaction, the first step is reduction of the GSH-RD by NADPH, and then the enzyme catalyzes the reduction of GSSG to form GSH. However, inhibition of the enzyme by tannic acid was not enhanced significantly in the presence of NADPH. As discussed above, inhibition of GSH-RD by tannic acid increased quickly with incubation time. The inhibition of GSH-RD by tannic acid after a 1-hr incubation almost reached maximum, even without preincubation with NADPH (Table 3). Therefore, preincubation with NADPH could not have a noticeable effect on the inhibition of GSH-RD by tannic acid.

Involvement of reactive oxygen species in the mechanism of inhibition of GSH-RD by dephinidin chloride and myricetin flavonoids has been described [11]. Superoxide anion radicals might react with certain flavonoids (e.g. myricetin) to form reactive intermediates, which, in turn, inhibited GSH-RD. However, the inhibition of GSH-RD by morin and quercetin remained the same under both hypoxic and aerobic conditions [11]. This suggested that the proposed oxygen-dependent mechanisms may work only for some individual flavonoids. Polyphenols have been

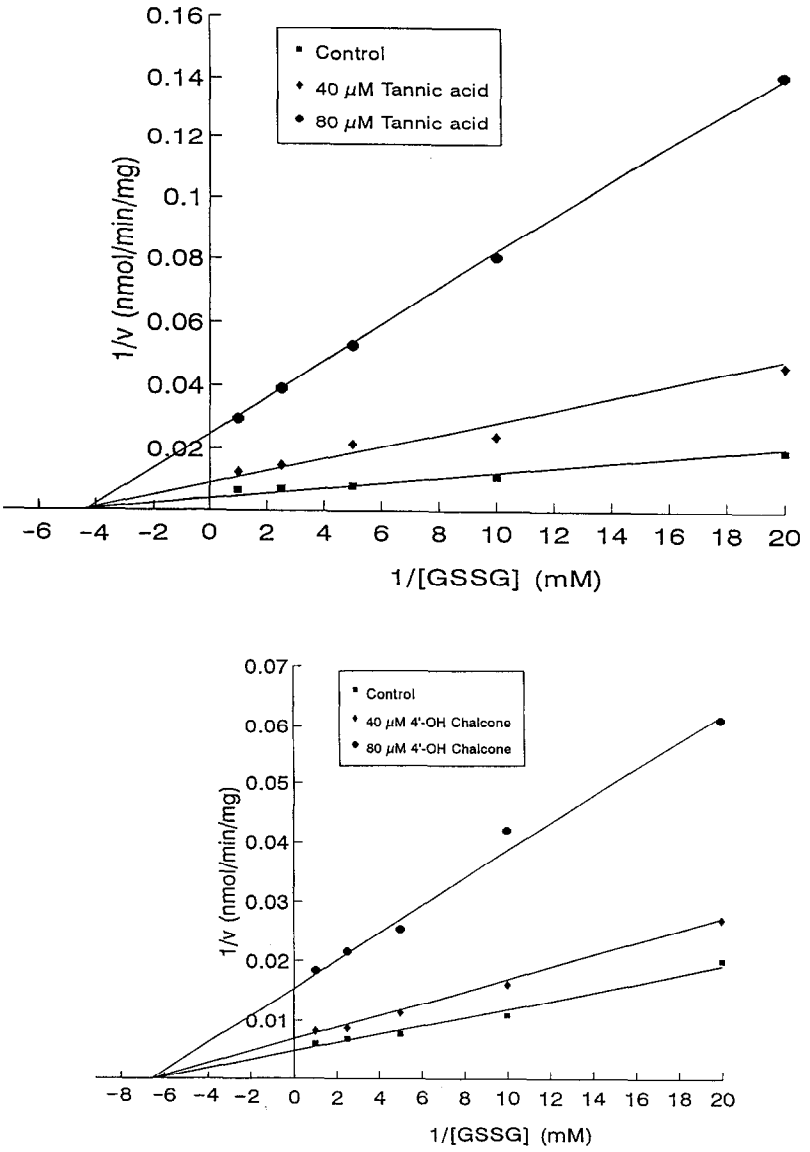


FIG. 3. Inhibitory kinetics of GSH-RD by 4'-hydroxychalcone and tannic acid. Experiments were carried out by various concentration of GSSG from 0.05 to 1 mM as described in Materials and Methods. Both tannic acid (top panel) and 4'-hydroxychalcone (bottom panel) showed non-competitive inhibition towards GSSG (V_{max} decreased; K_m remained unchanged). Data points are mean values of three separate experiments.

shown to complex with proteins through hydrogen and covalent bonds, causing precipitation [20]. This may be an oxygen-independent mechanism involved in the inhibition of GSH-RD by some polyphenols.

Drug resistance is a major problem in cancer chemotherapy. Many chemosensitizers were developed for sensitization of drug-resistant tumor cells. Verapamil, cyclosporin A, and its analogue, PCS 833, could reverse cellular multidrug resistance (MDR) by blocking P-glycoprotein-linked efflux of drugs [21, 22]. However, they were not effective in reversing MDR mediated by multidrug resistance-associated protein (MRP) [23]. Our previous studies have shown that polyphenols could sensitize human colon adenocarcinoma cells by inhibiting GSTs and transport of drug–glutathione conjugates [12–14]. In this *in vitro* study, polyphenols also inhibited glutathione reductase at micromolar concentrations. This inhibition of GSH-RD by plant polyphenols and a resulting low level of reduced GSH may decrease the detoxification of anticancer drugs by GSH conjugation and sensitize drug-resistant cells to these chemotherapeutic agents. The biological, pharmacological, and medicinal properties of these polyphenols have been studied extensively [24]. Quercetin and tangeretin have been shown to have preferential growth-inhibitory effects on tumor cells [25]. The antineoplastic properties of these polyphenols may be due to an increase of cellular cyclic AMP level [26], inhibition of protein kinase [27], and inhibition of DNA, RNA, and protein synthesis [28]. These effects of plant polyphenols may also contribute to sensitization of human tumor cells to anticancer drugs. As natural products found in many traditional herbal medicines [29] and in the human diet [30], these polyphenols should be more acceptable as chemosensitizers for cancer chemotherapy.

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References

1. Mannervik B and Danielson UH, Glutathione S-transferase: Structure and catalytic activity. *CRC Crit Rev Biochem* **23:** 283–337, 1988.
2. Tew KD, Glutathione-associated enzymes in anticancer drug resistance. *Cancer Res* **54:** 4313–4320, 1994.
3. Schecter RL, Alaoui-Jalami MA and Batist G, Glutathione S-transferases in chemotherapy resistance and carcinogenesis. *Biochem Cell Biol* **70:** 349–353, 1992.
4. Schneider E, Yamazaki H, Sinha BK and Cowan KH, Buthionine sulphoximine-mediated sensitisation of etoposide-resistant human breast cancer MCF7 cells overexpressing the multidrug resistance-associated protein involves increased drug accumulation. *Br J Cancer* **71:** 738–743, 1995.
5. Godwin AK, Meister A, O'Dwyer PJ, Huang CS, Hamilton TC and Anderson ME, High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis. *Proc Natl Acad Sci USA* **89:** 3070–3074, 1992.
6. Chen G and Waxman DJ, Identification of glutathione S-transferase as a determinant of 4-hydroperoxycyclophosphamide resistance in human breast cancer cells. *Biochem Pharmacol* **49:** 1691–1701, 1995.
7. Meister A and Anderson ME, Glutathione. *Annu Rev Biochem* **52:** 711–760, 1983.
8. Commandeur JNM, Stijntjes GJ and Vermeulen PE, Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Role in bioactivation and detoxication mechanisms of xenobiotics. *Pharmacol Rev* **47:** 271–330, 1995.
9. Colvin OM, Friedman HS, Gamcsik MP, Fenselau C and Hilton J, Role of glutathione in cellular resistance to alkylating agents. *Adv Enzyme Regul* **33:** 19–26, 1993.
10. Muller JG, Bucheler US and Krauth-Siegel RL, Glutathione reductase in human and murine lung tumors: High levels of mRNA and enzymatic activity. *Cell Mol Biol (Noisy-le-grand)* **39:** 389–396, 1993.
11. Elliott AJ, Scheiber SA, Thomas C and Pardini RS, Inhibition of glutathione reductase by flavonoids: A structure–activity study. *Biochem Pharmacol* **44:** 1603–1608, 1992.
12. Zhang K and Wong KP, Glutathione conjugation of chlorambucil: Measurement and modulation by plant polyphenols. *Biochem J*, **325:** 417–422, 1997.
13. Zhang K and Das NP, Inhibitory effects of plant polyphenols on rat liver glutathione S-transferases. *Biochem Pharmacol* **47:** 2063–2068, 1994.
14. Zhang K and Wong KP, Inhibition of the efflux of glutathione conjugate by plant polyphenols. *Biochem Pharmacol* **52:** 1631–1638, 1996.
15. Racker E, Glutathione reductase (liver and yeast). In: *Methods in Enzymology* (Eds. Colowick SP and Kaplan NO), Vol. II, pp. 722–725. Academic Press, New York, 1955.
16. Snedecor GW and Cochran WG, The comparison of two samples. *Statistical Methods* pp. 83–106. Iowa State University Press, Ames, IA, 1989.
17. Merlos M, Sanchez RM, Camarasa T and Adzet T, Flavonoids as inhibitors of rat liver cytosolic glutathione S-transferases. *Experientia* **47:** 616–619, 1991.
18. Kuppusamy UR and Das NP, Effects of flavonoids on several venom hyaluronidases. *Experientia* **47:** 1196–1200, 1991.
19. Kuppusamy UR and Das NP, Effects of flavonoids on cyclic AMP phosphodiesterase and lipid mobilization in rat adipocytes. *Biochem Pharmacol* **44:** 1307–1315, 1992.
20. Haslam E and Lilley TH, Interactions of natural phenols with macromolecules. In: *Plant Polyphenols in Biology and Medicine: Biochemical, Pharmacological, and Structure–Activity Relationships* (Eds. Cody V, Middleton E, Harborne JB and Beretz A), pp. 53–65. Alan R. Liss, New York, 1986.
21. Ford JM and Hait WN, Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol Rev* **42:** 155–199, 1990.
22. Keller RP, Altermatt HJ, Donatsch P, Zihlmann P, Laissue JA and Hiestand PC, Pharmacological interactions between the resistance-modifying cyclosporin SDZ PSC 833 and etoposide (VP 16-123) enhance *in vivo* cytostatic activity and toxicity. *Int J Cancer* **51:** 433–438, 1992.
23. Barrand MA, Rhodes T, Center MS and Twentyman PR, Chemosensitization and drug accumulation effects of cyclosporin A, PSC-833 and verapamil in human MDR large cell lung cancer cells expressing a 190k membrane protein distinct from P-glycoprotein. *Eur J Cancer* **29A:** 408–415, 1993.
24. Middleton E and Kandaswami C, The impact of plant flavonoids on mammalian biology: Implications for immunity, inflammation and cancer. In: *Flavonoids: Advances in Research Since 1986* (Ed. Harborne JB), pp. 619–652. Chapman & Hall, London, 1994.
25. Kandaswami C, Perkins E, Drzewiecki G, Soloniuk DS and Middleton E, Differential inhibition of proliferation of human squamous cell carcinoma, gliosarcoma and embryonic fibroblast-like lung cells in culture by plant flavonoids. *Anticancer Drugs* **3:** 525–530, 1992.
26. Graziani Y, Winikoff J and Chayoth R, Regulation of cyclic AMP level and lactic acid production in Ehrlich ascites tumor cells. *Biochim Biophys Acta* **497:** 499–505, 1977.
27. Cunningham BDM, Threadgill MD and Hickman HA, Synthesis and evaluation of substituted flavones as inhibitors of tyrosine protein kinase. *Br J Cancer* **56:** 207–213, 1987.
28. Graziani Y and Chayoth R, Regulation of cyclic AMP level and synthesis of DNA, RNA and protein by quercetin in Ehrlich ascites tumor cells. *Biochem Pharmacol* **28:** 397–403, 1979.
29. Haslam E, Lelly TH, Ya C, Martin R and Magnolato D, Traditional herbal medicine. The role of plant polyphenols. *Planta Med* **55:** 1–8, 1989.
30. Kuhnau J, The flavonoids: A class of semi-essential food components: The role in human nutrition. *World Rev Nutr Diet* **24:** 117–121, 1976.