Decrease of plasma and urinary oxidative metabolites of acetaminophen after consumption of watercress by human volunteers

To investigate the effect of the consumption of watercress *(Nasturtium officinale R. Br.)*, a cruciferous vegetable, on acetaminophen metabolism, the pharmacokinetics of acetaminophen and its metabolites were studied in a crossover trial of human volunteers. A single oral dose of acetaminophen (1 gm) was given 10 hours after ingestion of watercress homogenates (50 gm). In comparison with acetaminophen only, the ingestion of watercress resulted in a significant reduction in the area under the plasma cysteine acetaminophen (Cys-acetaminophen) concentration–time curve and in the peak plasma Cys-acetaminophen concentration by 28% ± 3% and by 21% ± 4% (mean ± SE; $n = 7$; $p < 0.005$), respectively. Correspondingly, the Cys-acetaminophen formation rate constant and Cys-acetaminophen formation fraction were decreased by 55% ± 9% and 52% ± 7% ($p < 0.01$), respectively. Consistent with the results obtained from the plasma, the total urinary excretion of Cys-acetaminophen in 24 hours was also reduced. A decrease of mercapturate acetaminophen, a Cys-acetaminophen metabolite, was also shown in the plasma and urine samples. However, the plasma pharmacokinetic processes and the urinary excretions of acetaminophen, acetaminophen glucuronide, and acetaminophen sulfate were not altered significantly by the watercress treatment. These results suggest that the consumption of watercress causes a decrease in the levels of oxidative metabolites of acetaminophen, probably due to inhibition of oxidative metabolism of this drug. (Clin Pharmacol Ther 1996;60:651-60.)

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Previous studies have shown that phenethyl isothiocyanate (PEITC), a component derived from watercress *(Nasturtium officinale R. Br.)*, inhibits 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)–induced lung carcinogenesis and *N*-nitrosobenzylmethylamine–induced esophageal tumors in laboratory animals.[1,2] This inhibitory action may be primarily due to inhibition of activation of the carcinogens by PEITC. In separate studies, PEITC has been shown to be an effective inhibitor of P4502E1, an enzyme involved in the metabolic activation of many low molecular weight toxicants and carcinogens.[3-5] These results suggest the possibility that PEITC could be used as a chemopreventive agent to lower the risk of chemically induced toxicity and carcinogenicity.

Acetaminophen is used widely as an analgesic and antipyretic agent. The major metabolic pathways of a therapeutic dose of acetaminophen in humans are glucuronidation and sulfation, which account for about 60% and 30% of its metabolism, respectively.[6] Only a small percentage of acetaminophen is oxidized to *N*-acetyl-*p*-benzoquinone imine (NAPQI),[7-9] which is believed to be the reactive metabolite leading to hepatotoxicity.[10-14] The activation of acetaminophen to NAPQI is known to be catalyzed by cytochromes P450 (P450) 2E1, 3A4, and 1A2 in

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humans.[15-17] With therapeutic doses of acetaminophen, NAPQI is effectively detoxified by conjugation with glutathione to form glutathione acetaminophen (GSH-acetaminophen), which is further converted to cysteine acetaminophen (Cys-acetaminophen) and then mercapturate acetaminophen (Mer-acetaminophen).[18]

It is known that carcinogens share many of the metabolic pathways with drugs in their biotransformation. For example, the activation of the tobacco-specific carcinogen NNK by P450 enzymes generates electrophilic intermediates.[19] This metabolic pattern is similar to P450-mediated activation of acetaminophen to the reactive intermediate NAPQI. Because of this similarity in oxidative metabolism, acetaminophen could be used as a model substrate for investigation of the effect of PEITC on xenobiotic metabolism, although there is no evidence that acetaminophen is associated with carcinogenesis.

Watercress is a cruciferous vegetable rich in gluconasturtiin, a glucosinolate precursor of PEITC.[20] Ingestion of watercress could result in the appearance of the PEITC metabolite *N*-acetylcysteine PEITC in human urine, suggesting that watercress could serve as a dietary source of PEITC.[21] Because PEITC inhibits xenobiotic metabolism of animals in vitro[3-5] and in vivo,[1,2] we investigated herein whether watercress consumption affects xenobiotic metabolism in humans. The pharmacokinetics of acetaminophen and its metabolites were studied in a crossover trial of human volunteers after a single oral dose of acetaminophen in the presence or absence of watercress.

MATERIAL AND METHODS

Materials. Acetaminophen tablets (Tylenol, 500 mg tablets) were obtained from McNeil Consumer Products Co. (Fort Washington, Pa.). The reagent grade acetaminophen standard was purchased from Sigma Chemical Co. (St. Louis, Mo.). Fresh watercress was purchased from a local supermarket. The standards of acetaminophen metabolites: Glu-acetaminophen, Sul-acetaminophen, GSH-acetaminophen, Cys-acetaminophen, and Mer-acetaminophen were provided by Dr. L. Ohannesian (McNeil Consumer Products Co. (Fort Washington, Pa.). The standards of acetaminophen metabolites 3-hydroxyacetaminophen and 3-methoxyacetaminophen were the gifts from Dr. S. D. Nelson (University of Washington, Seattle, Wash.). All other chemicals and solvents were of the highest grade materials commercially available.

Human study. Ten healthy volunteers (seven men and three women; age range, 23 to 48 years; weight range, 59 to 77 kg) gave written informed consent to the study, which was approved by the Institutional Review Board for Human Experimentation of Rutgers University (Piscataway, N.J.). The study was limited to subjects who were nonsmokers and who drank alcohol infrequently or not at all. Subjects were required to refrain from taking any medication or alcohol for 3 days before the study and during the study period of 2 weeks.

A crossover experimental design was used in this study. In the first experiment, volunteers were divided into two groups: four men in group A and three men in group B. Watercress was homogenized in water (1:1 by weight) for 2 to 3 minutes in a blender. Subjects in group A ingested watercress homogenates (equivalent to 50 gm watercress) at 10 pm and subjects in group B received water. The subjects in both groups fasted overnight. At approximately 8 am the next morning, subjects in both groups emptied their bladders just before taking acetaminophen (1 gm with 250 ml water). No food was ingested until 10 am. A Surflo intravenous catheter was inserted into a forearm vein and the blood samples (2 ml) were taken into a heparinized tube at 0, ¼, ½, ¾, 1¼, 3, 4, 6, 8, 10, and 12 hours after ingestion of acetaminophen. The blood samples were then centrifuged at 2000*g* for 10 minutes, and the resultant plasma samples were frozen at −70° C until analyzed. Urine samples were collected at the intervals of 0 to 1, 1 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 12, and 12 to 24 hours. The urine bottles containing urine sample were kept at −20° C after the collection. The urine samples were thawed within 2 days, the total volumes measured, and aliquots stored at −70° C until analyzed. Two weeks later, the treatments for groups A and B were switched, and the experiment was repeated as described above.

To confirm the results obtained from the first experiment, a second crossover experiment was conducted but only the urine samples were collected. In this experiment, four men in group A (the same subjects as the first experiment) received water and three women in group C ingested the watercress homogenates. After 2 weeks, the treatment before administration of acetaminophen in groups A and C was switched.

Analysis of acetaminophen and its metabolites in plasma and urine samples. For precipitating plasma proteins, 20 μl of a 2.5% zinc sulfate solution in water was added to 100 μl plasma in a polypro-

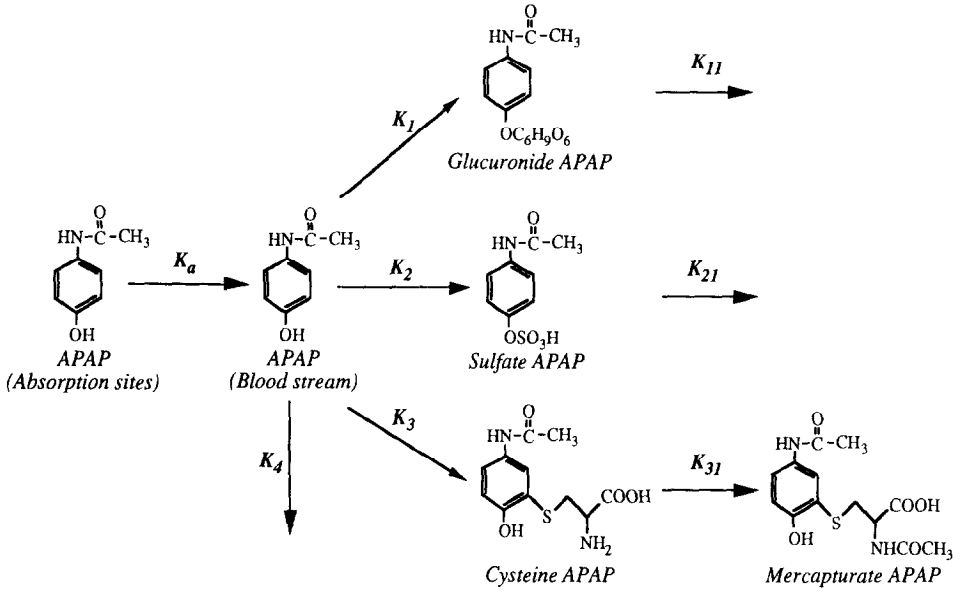


Fig. 1. Representative model for acetaminophen (APAP) and its metabolites pharmacokinetic analysis.

pylene microcentrifuge tube. The sample was vortex-mixed and centrifuged at 16,000*g* for 3 minutes. The supernatant (20 μl) was injected onto the HPLC. The urine samples were diluted 10 times with water, and 100 μl of the diluted samples were mixed with 20 μl of a 2.5% zinc sulfate solution. After centrifugation, 20 μl of the supernatant was injected onto the HPLC.

The HPLC system (Waters 840) consisted of a model 510 pump, a model 712 WISP autoinjector, a model 490E programmable multiwavelength detector (ultraviolet) set at 240 nm (for the analysis of acetaminophen, glucuronide and sulfate conjugates), a model 320 electrochemical detector set at a potential voltage of 0.7 V (for the analysis of oxidative metabolites), and a Waters model 380 digital professional data system. Separation was accomplished at ambient temperature (23° ± 1° C) on a 5 μm Spherisorb LC-ABZ column (250 mm × 4.6 mm internal diameter; Supelco, Inc., Bellefonte, Pa.) equipped with a direct-connect guard column. After the sample injection, the column was eluted with a mobile phase solution (93% water, 7% acetonitrile, and 0.15% trifluoric acid, vol/vol/vol) at a flow rate of 1.8 ml/min for 11 minutes. Then the column was washed with a mixture of water and acetonitrile (22:78, vol/vol) for 6 minutes and reequilibrated with the mobile phase solution for another 13 minutes. The limit of detection was estimated to be 0.005 μg/ml for Cys-acetaminophen, GSH-acetaminophen, and Mer-acetaminophen monitored by the electrochemical detector, and 0.25 μg/ml for acetaminophen, Glu-acetaminophen, and Sul-acetaminophen monitored by the ultraviolet detector, respectively. The amounts of acetaminophen and its metabolites were determined from the standard curves constructed from a mixture of the standards in each run. All data were obtained from duplicate measurements.

Pharmacokinetic analysis. The best fits for the plasma concentration–time data of acetaminophen, Glu-acetaminophen, Sul-acetaminophen, and Cys-acetaminophen were achieved by the one-compartment model as accessed by correlation coefficient (r^2), the Akaike information criterion (AIC), and Schwartz criterion (SC).[22,23] Then a combined pharmacokinetic model (first-order absorption and elimination of acetaminophen and the first-order formation and elimination of its metabolites) was established to describe the time courses of acetaminophen, Glu-acetaminophen, Sul-acetaminophen, and Cys-acetaminophen after an oral administration of acetaminophen in human subjects (Fig. 1). The concentration–time data sets for acetaminophen, Glu-acetaminophen, Sul-acetaminophen, and Cys-acetaminophen from individual subjects were simultaneously fitted according to equations 6 to 9, as shown in Appendix, to obtain

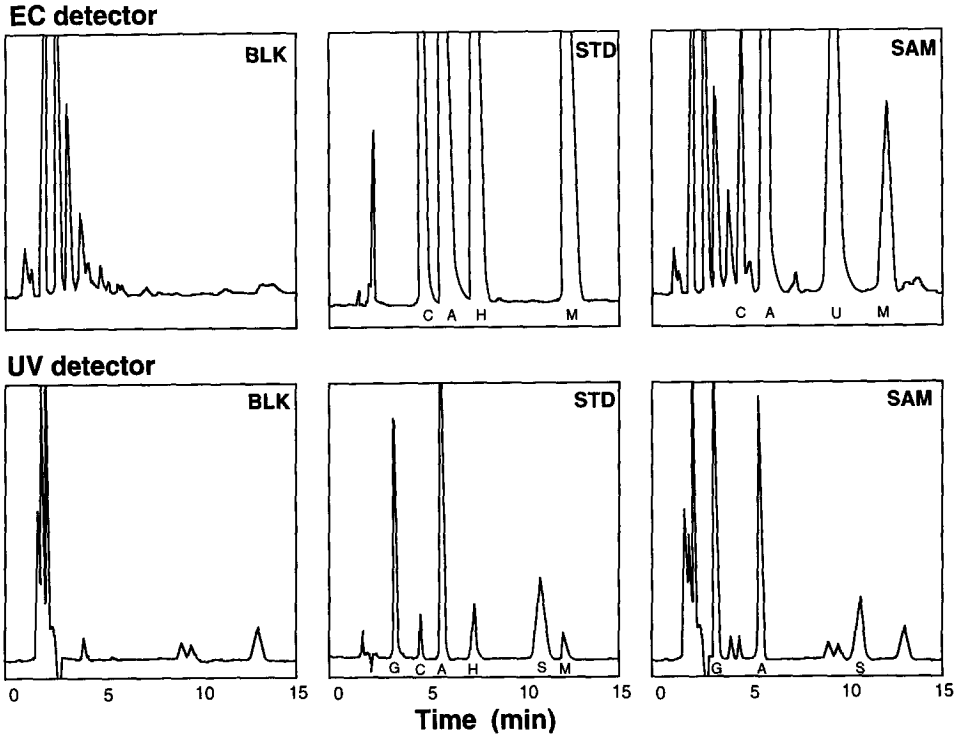


Fig. 2. Chromatograms of blank plasma (BLK), standard (STD), and a plasma sample (SAM) obtained 3 hours after an oral dose (1 gm) of acetaminophen. Retention time of peaks: C, Cys-acetaminophen; A, acetaminophen; H, GSH-acetaminophen; U, unknown metabolite; M, Mer-acetaminophen; G, Glu-acetaminophen; S, Sul-acetaminophen. EC, Electrochemical; UV, ultraviolet.

the primary pharmacokinetic parameters with use of the PCNONLIN software package (Version 4.2, ClinTrials, Lexington, Ky.). Data for model-fitting were iteratively reweighted by modulating the reciprocal of the predicted concentrations. Appropriate fitting was based on achieving a result with a minimal sum of squared residuals, the lowest possible standard deviations of parameter estimates, and unbiased distribution patterns of residuals of estimates of observed versus predicted concentrations. The secondary pharmacokinetic parameters were calculated according to Gibaldi and Perrier.[24]

Statistical analysis. Data are expressed as the mean ± SE. The Student paired *t* test was used to access the significant difference between the control and the watercress treatment groups.

RESULTS

HPLC profile for acetaminophen and its metabolites. The retention times of Glu-acetaminophen, acetaminophen, and Sul-acetaminophen were 3.4, 5.9, and 11.1 minutes, respectively, with the ultraviolet detector, and the retention times of Cys-acetaminophen, GSH-acetaminophen, and Mer-acetaminophen were 4.9, 7.8, and 13.1 minutes, respectively, with the electrochemical detector. However, the GSH-acetaminophen in the plasma and urine samples was undetectable by the HPLC method used. Typical chromatograms of the standard and plasma sample are presented in Fig. 2. In addition to the above metabolites, a new peak was observed at 9.6 minutes on the electrochemical detector due to acetaminophen administration. Because it gave a signal in the electrochemical detector, it is likely to be an oxidative metabolite. This metabolite peak height was not affected by watercress pretreatment during the sampling period. The standards of 3-hydroxyacetaminophen and 3-methoxyacetaminophen had retention times of 3.9 and 4.3 minutes, respectively, in the electrochemical detection. However, these signals were undetectable in the plasma and urine samples, suggesting that 3-hydroxylation of acetaminophen is not a significant pathway.

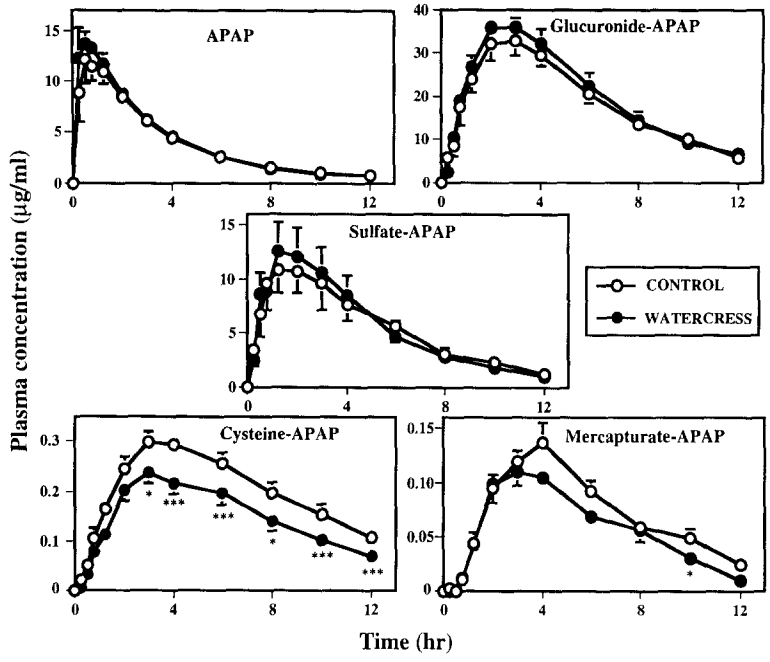


Fig. 3. Plasma concentration versus time profiles of acetaminophen (APAP) and its metabolites after a single oral dose (1 gm) of acetaminophen in human subjects. Each *data point* represents the mean ± SE of seven individual subjects. $^{*}p < 0.05$; $^{***}p < 0.005$.

Effect of watercress on plasma acetaminophen and its metabolites. The subjects did not report any side effects from the administration of watercress, acetaminophen, or acetaminophen after watercress treatment. The effects of watercress on the plasma concentrations of acetaminophen, Glu-acetaminophen, Sul-acetaminophen, Cys-acetaminophen, and Mer-acetaminophen are shown in Fig. 3. A significant decrease was observed in the oxidative metabolite of Cys-acetaminophen at 3, 4, 6, 8, and 12 hours after the acetaminophen administration, but acetaminophen, Glu-acetaminophen, and Sul-acetaminophen were not significantly changed at all time points measured. A slight reduction of the plasma concentration of Mer-acetaminophen, a metabolite of Cys-acetaminophen, was also observed.

The effect of watercress pretreatment on the pharmacokinetic parameters of acetaminophen and its metabolites is shown in Table I. The watercress treatment did not cause a significant change in acetaminophen pharmacokinetic parameters, K_a, K, CL_A, AUC_A, and $C_{max,A}$. The major metabolic pathways, the glucuronidation and sulfation of acetaminophen, were also not altered by the watercress treatment because all of the pharmacokinetic parameters regarding Glu-acetaminophen and Sul-acetaminophen disposition remained unchanged. However, the formation of Cys-acetaminophen was significantly decreased in terms of K_3 (mean ± SE; by 55% ± 9%; $n = 7$; $p < 0.01$), AUC_C (by 28% ± 3%; $p < 0.005$), $C_{max,C}$ (by 21% ± 4%; $p < 0.005$), and fraction of conversion f_{mC} (K_3/K) (by 52% ± 7%, $p < 0.01$). A 20% decrease in the AUC_M of Mer-acetaminophen was also shown ($p < 0.05$). All of the parameters regarding the elimination for all metabolites (K_{11}, K_{21}, and K_{31}) were not changed significantly (Table I).

Effect of watercress on the urinary excretion of acetaminophen and its metabolites. The total amount of excretions within 24 hours for acetaminophen, Glu-acetaminophen, and Sul-acetaminophen were not altered significantly in both of experiments 1 and 2 (Tables II and III). However, the excretion of oxidation-derived metabolites was decreased in terms of fraction of excretion: Cys-acetaminophen and Mer-acetaminophen were decreased by 30% ± 6% and 24% ± 4%, respectively, in experiment 1 (Table II) and were decreased by 40% ± 6% and 38% ± 4%, respectively, in experiment 2 (Table

Table I. Effect of watercress on plasma pharmacokinetic parameters of acetaminophen and its metabolites

Parameter	*Control*	*Watercress*
APAP		
K_a (hr^{-1})	0.25 ± 0.02	0.28 ± 0.02
K (hr^{-1})	3.88 ± 1.01	2.85 ± 0.30
CL_A (L/hr)	20.2 ± 1.5	19.7 ± 1.2
AUC_A (mg · hr/L)	51.3 ± 4.0	51.4 ± 3.2
$C_{max,A}$ (μg/ml)	14.6 ± 1.8	15.8 ± 1.6
Glu-APAP		
K_1 (hr^{-1})	2.14 ± 0.51	1.74 ± 0.16
K_{11} (hr^{-1})	0.82 ± 0.16	0.69 ± 0.09
AUC_G (mg · hr/L)	236.1 ± 24.9	250.8 ± 23.2
$C_{max,G}$ (μg/ml)	34.2 ± 3.9	38.0 ± 2.6
$f_{m,G}$ (%)	58.0 ± 5.6	63.1 ± 4.6
Sul-APAP		
K_2 (hr^{-1})	1.32 ± 0.48	0.88 ± 0.22
K_{21} (hr^{-1})	3.84 ± 1.35	2.94 ± 0.88
AUC_S (mg · hr/L)	67.6 ± 14.9	69.8 ± 14.8
$C_{max,S}$ (μg/ml)	11.8 ± 2.3	13.7 ± 2.6
$f_{m,S}$ (%)	30.9 ± 5.7	28.7 ± 4.8
Cys-APAP		
K_3 (hr^{-1})	0.21 ± 0.05	0.08 ± 0.01**
K_{31} (hr^{-1})	0.36 ± 0.06	0.34 ± 0.04
AUC_C (mg · hr/L)	2.8 ± 0.2	2.1 ± 0.2***
$C_{max,C}$ (μg/ml)	0.31 ± 0.02	0.25 ± 0.02***
$f_{m,C}$ (%)	6.1 ± 0.8	2.8 ± 0.4**
Mer-APAP		
AUC_M (mg · hr/L)	0.89 ± 0.10	0.71 ± 0.07*
$C_{max,M}$ (μg/ml)	0.14 ± 0.02	0.12 ± 0.01

Data are expressed as mean values ± SE of seven subjects.

APAP, Acetaminophen; K_a, APAP absorption rate constant; K, APAP elimination rate constant; CL, apparent clearance; AUC, area under the plasma concentration–time curve; C_{max}, plasma peak concentration; K_1, K_2, and K_3, formation rate constant, respectively, for Glu-APAP, Sul-APAP, and Cys-APAP; K_{11}, K_{21}, and K_{31}, elimination rate constant, respectively, for Glu-APAP, Sul-APAP, and Cys-APAP; f_m, fraction of metabolite formation; the subscript capital letters of A, G, S, C, and M indicate the labeled parameter is regarded to APAP, Glu-APAP, Sul-APAP, Cys-APAP, and Mer-APAP, respectively.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.005$.

III). All subjects had consistently lower fraction of excretion for Cys-acetaminophen and Mer-acetaminophen after the watercress treatment, but the fractions of excretion for acetaminophen, Glu-acetaminophen, and Sul-acetaminophen were unchanged. Four of the 10 volunteers participated in both experiments 1 and 2. A consistent reduction of the urinary excretions by the ingestion of watercress was observed in the four subjects in the two experiments (Fig. 4). When the results from experiments 1 and 2 were combined, a significant decrease in the excretion rate was observed for Cys-acetaminophen in the intervals of 4 to 6 and 6 to 8 hours and for Mer-acetaminophen in the intervals of 4 to 6, 6 to 8, and 8 to 12 hours (Fig. 5). However, the excretion rates of acetaminophen, Glu-acetaminophen, and Sul-acetaminophen were not changed (Fig. 5).

DISCUSSION

Cys-acetaminophen and Mer-acetaminophen are hydrolysis products of oxidation-derived metabolite GSH-acetaminophen (the conjugate of NAPQI with GSH). In this study, we used the formations of Cys-acetaminophen and Mer-acetaminophen as indicators of oxidative metabolism of acetaminophen. This study shows that a single watercress ingestion inhibits the formations of Cys-acetaminophen and Mer-acetaminophen. In plasma, the formations of Cys-acetaminophen and Mer-acetaminophen were decreased significantly by the watercress treatment (Table I and Fig. 3). The inhibitory effect was further confirmed by the analysis of the urine samples: Cys-acetaminophen and Mer-acetaminophen were decreased consistently in all the tested volunteers and the repeat-tested volunteers after the consumption of watercress (Tables II and III; Figs. 4 and 5). However, the levels of acetaminophen, acetaminophen glucuronide, and acetaminophen sulfate were not altered significantly in both the plasma and urine samples. These results are consistent with the concept that watercress consumption suppresses the oxidative pathway of acetaminophen, without changing its conjugations with glucuronide and sulfate. An alternative interpretation is that watercress consumption resulted in an inhibition of GSH-acetaminophen formation; for example, by the quenching of NAPQI with nucleophilic species.

Watercress is rich in gluconasturtiin, a PEITC precursor. Previous results have shown the appearance of PEITC metabolite, *N*-acetylcysteine PEITC, in the urine of human volunteers who ingested raw watercress.[21] In previous studies with mice, treatment with PEITC resulted in a significant decreases in the levels of GSH-acetaminophen, Cys-acetaminophen, and Mer-acetaminophen in plasma and urine (Li et al. Unpublished results, 1996). It is possible that PEITC is responsible for a significant part of the presently observed decreases of Cys-acetaminophen and Mer-acetaminophen. Oxidation of acetaminophen is known to be activated mainly by P450s 2E1, 3A4, and 1A2 in humans[15-17] to form NAPQI. Considering K_m values, turnover rate, and the abundance of the enzymes,[16] P4502E1 and P4503A4 are thought to be

Table II. Effect of watercress on urinary excretions of acetaminophen and its metabolites (experiment 1)

	Control	*Watercress*	*Control*	*Watercress*
	Excreted amount (μmol)		*Fraction of excretion (%)*	
APAP	122.7 ± 17.1	120.6 ± 14.6	2.5 ± 0.4	2.4 ± 0.4
Glu-APAP	3362.1 ± 642.2	3479.5 ± 473.5	62.0 ± 3.3	63.8 ± 2.6
Sul-APAP	1648.6 ± 251.0	1647.5 ± 192.4	31.6 ± 2.9	31.0 ± 2.3
Cys-APAP	102.4 ± 17.1	72.8 ± 10.8	2.0 ± 0.2	1.3 ± 0.1**
Mer-APAP	97.85 ± 18.1	80.2 ± 17.0	1.9 ± 0.2	1.4 ± 0.2**
TOTAL	5333.7 ± 879.4	5400.6 ± 634.3	100	100

Acetaminophen was given orally to volunteers with a dose of 1 gm (6614 μmol). The total urinary recovery for APAP and its metabolites was 81% and 82% in the control and watercress groups, respectively.
Data are expressed as mean values ± SE (n = 7).
**$p < 0.01$.

Table III. Effect of watercress on urinary excretions of acetaminophen and its metabolites (experiment 2)

	Control	*Watercress*	*Control*	*Watercress*
	Excreted amount (μmol)		*Fraction of excretion (%)*	
APAP	178.1 ± 28.8	145.6 ± 26.5	3.3 ± 0.5	2.9 ± 0.5
Glu-APAP	3072.3 ± 291.0	3078.2 ± 355.9	56.6 ± 1.6	59.8 ± 2.1
Sul-APAP	1884.1 ± 123.1	1737.5 ± 164.4	35.5 ± 1.6	34.6 ± 1.9
Cys-APAP	115.7 ± 14.6	67.4 ± 12.2**	2.2 ± 0.2	1.3 ± 0.1***
Mer-APAP	131.8 ± 23.4	76.4 ± 12.2**	2.4 ± 0.4	1.5 ± 0.2**
TOTAL	5382.0 ± 419.4	5105.0 ± 512.0	100	100

Acetaminophen was given orally to volunteers with a dose of 1 gm (6614 μmol). The total urinary recovery for APAP and its metabolites was 81% and 77% in the control and watercress groups, respectively.
Data are expressed as mean values ± SE (n = 7).
**$p < 0.01$; ***$p < 0.005$.

more important than P4501A2. Among the three proteins, P4502E1 is the enzyme most susceptible to the inhibition by PEITC in the liver microsomes of mice (Li et al. Unpublished results, 1996) and rats[4] both in vitro and in vivo. If the results observed in the present study are indeed due to the blockage of acetaminophen oxidation by PEITC, then inhibition of P4502E1 is a likely mechanism. However, the contribution from other constituents of watercress by other mechanisms to the presently observed decrease of Cys-acetaminophen and Mer-acetaminophen could not be excluded.

In a simple design, a single dose of watercress was used in this study. Each individual consumed 50 gm of fresh watercress, which is equivalent to a total intake of approximate 13 mg of PEITC per person.[21] The watercress was ingested 10 hours before acetaminophen administration, and this was designed (1) to avoid interference of acetaminophen absorption by watercress, and (2) because the liver peak level of PEITC is achieved 6 to 8 hours after PEITC administration to the mice,[25] the time to achieve maximum inhibition after ingestion of watercress was expected to be longer than 6 to 8 hours in human subjects. Previous studies indicated that PEITC administration to rats could slightly induce the activity of rat liver microsomal UDP-glucuronosyltransferase.[4] The induction of glucuronidation of acetaminophen was not observed under our experimental conditions.

Although the therapeutic dose of acetaminophen is usually safe, the risk for hepatotoxicity is enhanced by alcohol consumption or intake of drugs such as isoniazid,[26-28] possibly due to the induction of P4502E1. The results of the present study, either because of the inhibition of oxidative metabolism of acetaminophen or quenching of NAPQI, suggest that watercress ingestion may be helpful to decrease the burden of acetaminophen for hepatotoxicity. Watercress consumption may also inhibit the activation of environmental carcinogens. Such an inhibitory effect on the oxidation of a potent tobacco-specific carcinogen, NNK, has been shown recently.[29]

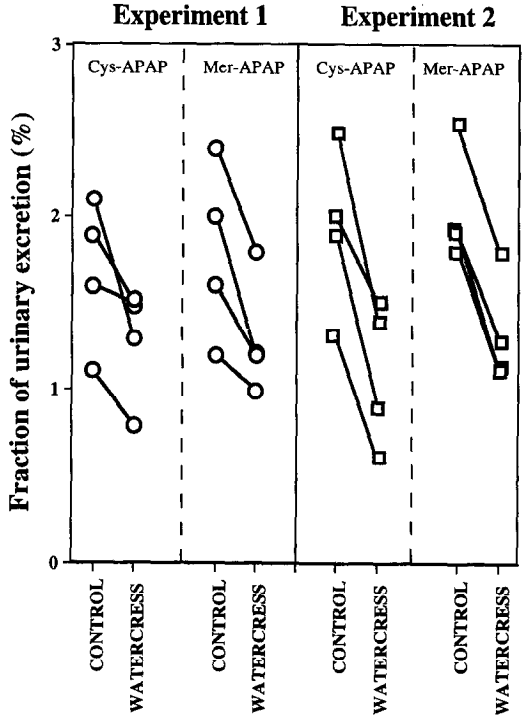


Fig. 4. Comparison of the effect of watercress on fraction of urinary excretion of cysteine-acetaminophen (Cys-APAP) and mercapturate-acetaminophen (Mer-APAP) after a single oral dose (1 gm) of acetaminophen in four human subjects participating in both experiments 1 and 2.

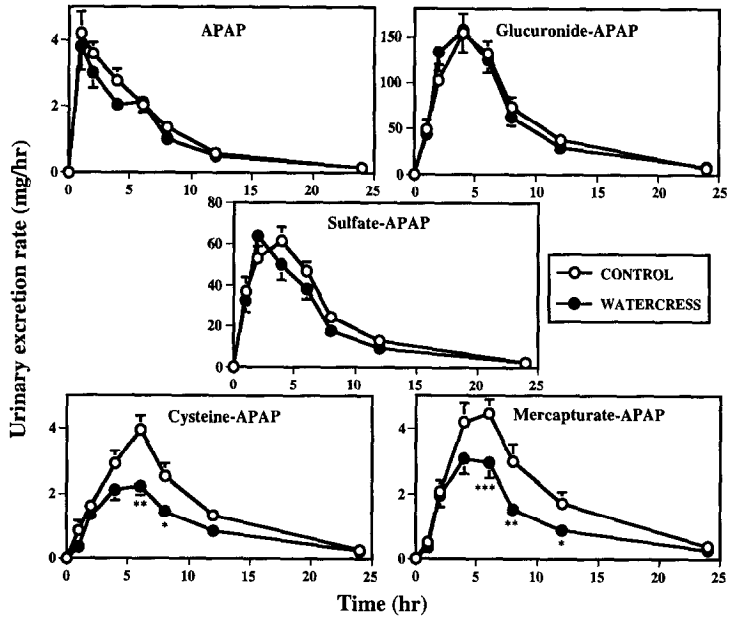


Fig. 5. Urinary excretion rate versus time profiles of acetaminophen (APAP) and its metabolites after a single oral dose (1 gm) of acetaminophen in human subjects. Each *data point* represents the mean ± SE of 14 measurements obtained from 10 subjects in experiments 1 and 2. $*p < 0.05$; $**p < 0.01$; $***p < 0.005$.

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APPENDIX

Based on the model in Fig. 1, the APAP absorption and elimination were described by the following:

$$\frac{dA_a}{dt} = -k_a \cdot A_a \quad (1)$$

and

$$\frac{dA}{dt} = K_a \cdot A_a - K \cdot A \quad (2)$$

The Glu-acetaminophen formation and elimination were described by the equation:

$$\frac{dG}{dt} = K_1 \cdot A - K_{11} \cdot G \quad (3)$$

The Sul-acetaminophen formation and elimination were described by the equation:

$$\frac{dS}{dt} = K_2 \cdot A - K_{21} \cdot S \quad (4)$$

and the Cys-acetaminophen formation and elimination were described by the equation:

$$\frac{dC}{dt} = K_3 \cdot A - K_{31} \cdot C \quad (5)$$

in which $K = K_1 + K_2 + K_3 + K_4$; A_a indicates the amount of acetaminophen in absorption site; A, G, S, and C indicate the amount of acetaminophen, Glu-acetaminophen, Sul-acetaminophen, and Cys-acetaminophen, respectively.

After Laplace transformation, equations 1 through 5 gave the following:

$$C'_A = \frac{K_a F A_0}{(K_a - K)V_A}(e^{-Kt} - e^{-K_a t}) \quad (6)$$

$$C'_G = \frac{K_1 K_a F A_0}{V_G}\left\{\frac{e^{-Kt}}{(K - K_a)(K - K_{11})} + \frac{e^{-K_a t}}{(K_a - K)(K_a - K_{11})} + \frac{e^{-K_{11} t}}{(K_{11} - K_a)(K_{11} - K)}\right\} \quad (7)$$

$$C'_S = \frac{K_2 K_a F A_0}{V_S}\left\{\frac{e^{-Kt}}{(K - K_a)(K - K_{21})} + \frac{e^{-K_a t}}{(K_a - K)(K_a - K_{21})} + \frac{e^{-K_{21} t}}{(K_{21} - K_a)(K_{21} - K)}\right\} \quad (8)$$

$$C'_C = \frac{K_3 K_a F A_0}{V_C}\left\{\frac{e^{-Kt}}{(K - K_a)(K - K_{31})} + \frac{e^{-K_a t}}{(K_a - K)(K_a - K_{31})} + \frac{e^{-K_{31} t}}{(K_{31} - K_a)(K_{31} - K)}\right\} \quad (9)$$

in which C′ was the concentration; F was the fraction of absorption (assuming F = 1); A_0 was the dose; V was the apparent distribution volume; the subscript capital letters of A, G, S, and C indicate that the labeled parameter is regarded to acetaminophen, Glu-acetaminophen, Sul-acetaminophen, and Cys-acetaminophen, respectively. Definitions of other parameters are described in the *legend* of Table I.