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Research Paper

Pharmacokinetics of Dietary Phenethyl Isothiocyanate in Rats

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Purpose. Phenethyl isothiocyanate (PEITC) is a dietary component present in cruciferous vegetables and reported to have chemopreventive properties. Previous reports of PEITC pharmacokinetics have measured total ITC (PEITC and its metabolites) in plasma. Our objective was to examine the dose-dependent pharmacokinetics and oral bioavailability of unchanged PEITC, as well as its pH- and temperature-dependent stability and its serum protein binding.

Methods. Stability was studied at different pH values at room temperature and 4°C. Protein binding was determined by equilibrium dialysis. For the pharmacokinetics study, male Sprague–Dawley rats were administered with PEITC at doses of 2, 10, 100, or 400 μmol/kg intravenously or 10 or 100 μmol/kg orally. Plasma samples were analyzed by liquid chromatography–tandem mass spectrometry. Pharmacokinetic analysis was conducted by WinNonlin and ADAPT II.

Results. Phenethyl isothiocyanate was stable in aqueous buffers at pH 7.4 with half-lives of 56.1 and 108 h at room temperature and 4°C, respectively. The free fraction of PEITC in rat serum was 0.019. The clearance (Cl) at a low dose of PEITC (2 μmol/kg) was 0.70 ± 0.17 L h^{-1} kg^{-1} with an apparent volume of distribution (V_{ss}) of 1.94 ± 0.42 L/kg. At higher doses, Cl tended to decrease, whereas V_{ss} increased. Oral bioavailability of PEITC was 115 and 93% at doses of 10 and 100 μmol/kg, respectively. A three-compartment model with Michaelis–Menten elimination and distribution was found to best characterize the plasma concentration profiles.

Conclusions. Phenethyl isothiocyanate is stable in biological samples, with increased stability under refrigerated conditions. It has high oral bioavailability, low clearance, and high protein binding in rats; nonlinear elimination and distribution occur following the administration of high doses. This investigation represents the first report of the pharmacokinetics of dietary PEITC.

KEY WORDS: bioavailability; PEITC; pharmacokinetics; phenethyl isothiocyanate; protein binding; rats; stability.

INTRODUCTION

Isothiocyanates (ITCs) occur widely as conjugates in the genus *Brassica* of cruciferous vegetables (e.g., cabbage, cauliflower, brussels sprouts, watercress, broccoli, kale) and the genus Raphanus (radishes and daikons) (1). They are released by the action of myrosinase (β-thioglucoside glucohydrolase) after plant cells are damaged, such as from cutting and chewing, or by hydrolysis in the intestinal tract by microflora (1,2). More than 25 natural and synthetic ITCs have been shown to block chemical carcinogenesis effectively (3). Isothiocyanates are widely consumed by humans, and it is estimated that human consumption of glucosinolates are as high as 300 mg/day (3). Phenethyl isothiocyanate (PEITC; Fig. 1) is one of the extensively studied ITCs and has been launched into phase I clinical trials as a lung cancer preventive agent in smokers and ex-smokers (4). It has been demonstrated to have effective chemoprevention activity for a wide variety of tumors, and no apparent toxicity has been observed in animal models (5). It has been long recognized that ITCs can inhibit phase I enzymes, including cytochrome P450 enzymes, to convert procarcinogens to highly reactive electrophilic carcinogens that can form DNA adducts; they also induce phase II enzymes, including glutathione *S*-transferase and quinone reductase, to inactivate carcinogens and promote their excretion (6). More recently, it has been found that ITCs could inhibit the cell cycle and induce apoptotic cell death (7–9).

The metabolism of ITCs has been studied in mice, rats, guinea pigs, and rabbits (10–14). In rats, ITCs having the RCH_2–N=C=S residue, including PEITC, have been found

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ABBREVIATIONS: AUC, area under the plasma concentration *vs.* time curve; BCRP, breast cancer resistance protein; C_{max}, maximal plasma concentration; Cl, clearance; *F*, bioavailability; f_u, the unbound fraction; ITC, isothiocyanate; i.v., intravenous; k_a, absorption rate constant; k_d, degradation rate constant; MRP, multidrug resistance-associated protein; PEITC, phenethyl isothiocyanate; PEITC-SG, glutathione conjugate of PEITC; RT, room temperature; t_{max}, time to reach C_{max}; $t_{1/2}$, elimination half-life; $t_{1/2,d}$, degradation half-life; V_{ss}, volume of distribution.

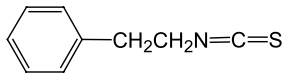

Fig. 1. The chemical structure of phenethyl isothiocyanate (PEITC).

to conjugate with glutathione first, catalyzed by glutathione *S*-transferases; the glutathione conjugate undergoes further metabolism to form a mercapturic acid conjugate (*N*-acetylcysteine conjugate) (12). In guinea pigs and rabbits, a cyclic mercaptopyruvate conjugate is the major metabolite (11), whereas mice excrete both the cyclic mercaptopyruvate and the *N*-acetylcysteine conjugate of PEITC, with the former form predominating (14). In humans, PEITC also forms a mercapturic acid conjugate, similar to the metabolite profile seen in rats (12,15). After ingestion of watercress, a dietary source of PEITC, a dose-dependent urinary excretion of the PEITC mercapturic acid conjugate was observed (16).

Knowledge regarding the disposition and excretion of PEITC is limited, although the pharmacokinetics of ^{14}C-PEITC in rats and mice after oral dose administration has been investigated. When administered to mice by gavage at a dose of 5 μmol, ^{14}C-PEITC was readily absorbed and distributed to all major tissues, and approximately 50% of the total radioactivity was excreted within 24 h (14). A study in rats showed similar tissue distribution, and whole blood ^{14}C radiolabel exhibited a disposition profile described by a two-compartment model with a t_{max} (time to reach maximal plasma concentration) of 2.9 h and an elimination half-life ($t_{1/2}$) of 21.7 h after an oral dose of 50 μmol/kg (17). However, measurement of total radioactivity does not reflect the pharmacokinetic behavior of the parent compound PEITC, but that of the parent drug and any metabolite containing the radiolabel. Phenethyl isothiocyanate has been used in phase I clinical trials to evaluate its safety and toxicity in healthy individuals, and the pharmacokinetic profiles of total ITC have been examined using a high-performance liquid chromatography (HPLC)-based cyclocondensation approach; PEITC, PEITC conjugates, as well as other ITCs or dithiocarbamates would be detected in this assay (4,18). No study has specifically determined the plasma concentration profiles of unchanged PEITC. However, characterization of the pharmacokinetics of PEITC is important to understand the *in vivo* concentration–effect and concentration–toxicity relationships and to design dosing regimens. Furthermore, the oral bioavailability of PEITC has not been determined, despite the facts that PEITC is widely present in the human diet and has often been dosed orally in most *in vivo* studies to investigate its chemopreventive activity.

Our laboratory has demonstrated that certain dietary ITCs inhibit the P-glycoprotein- (P-gp-) and multidrug resistance-associated protein 1- (MRP1-) mediated efflux of daunomycin and vinblastine in multidrug resistance cancer cells (19,20). More recently, we found that ITCs are inhibitors of breast cancer resistance protein (BCRP), a newly discovered ATP-binding cassette transporter, and 10- or 30-μM concentrations of ITCs could inhibit BCRP significantly (21). Because of the high expression of BCRP, MRP2, and P-gp in the human intestine as well as in human liver (22,23), ITCs may play a role in food–drug interactions by affecting the absorption and elimination of substrates for these transporters. Therefore, knowing the *in vivo* concentration ranges, oral bioavailability and pharmacokinetics of ITCs are important in our understanding of the effects of ITCs in food–drug interactions and multidrug resistance.

The present study examines the dose-dependent pharmacokinetics and oral bioavailability of PEITC in the rat animal model. This type of information cannot be obtained in humans because it requires intravenous (i.v.) administration of PEITC. The rat was used as the animal model because the metabolic disposition of PEITC is similar in rats and humans. We first examined the pH-dependent stability and serum protein binding of PEITC. Information regarding the stability of PEITC is essential for establishing sample-handling procedures for the pharmacokinetic study. We then determined the dose-dependent pharmacokinetics of PEITC in rats following oral and i.v. administration. Finally, a compartmental model was established to characterize the plasma concentration-time profiles obtained.

MATERIALS AND METHODS

Materials

Phenethyl isothiocyanate, hydroxypropyl-β-cyclodextrin, and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1,1,2,2-$^{2}H_4$-PEITC was synthesized and characterized in our laboratory as described previously (24). ^{14}C-PEITC was synthesized in our laboratory according to Conaway *et al.* (17), with specific activity of 0.04994 mCi/mg and concentration of 1 mCi/mL. All the solvents used for HPLC and liquid chromatography–tandem mass spectrometry (LC/MS/MS) analysis were HPLC grade and were purchased from Fisher Scientific (Springfield, NJ, USA).

Stability of Phenethyl Isothiocyanate

A universal buffer of citrate–phosphate–borate/HCl (Teorell & Stenhagen) was used to study the stability of PEITC. The buffer solutions, with pH of 3.0, 5.0, 7.4, 8.4, 9.3, and 10.1, were spiked with a 0.1 mM PEITC (in acetonitrile). All samples were left at room temperature (RT) and analyzed at various times throughout a 120-h period using an HPLC assay (24). The effect of storage temperature on the stability of PEITC was also determined. The degradation rate constant (k_d) was calculated from the slope of the degradation plot [log (% remaining) *vs.* time], and the degradation half-life ($t_{1/2,\ d}$) was calculated using the equation $t_{1/2,\ d} = \ln 2/k_d$.

Serum Protein Binding of Phenethyl Isothiocyanate

Protein binding measurements were determined by equilibrium dialysis using Spectra/Por Dialysis Tubing (Rancho Dominguez, CA, USA) with a 12,000–14,000 molecular weight cutoff at 37°C. Equal volumes (0.36 mL) of 0.13 M phosphate buffer (pH 7.4) and rat (Harlan Sprague–Dawley, Indianapolis, IN, USA) serum were added to each side of the dialysis chamber. ^{14}C-PEITC was added to the rat serum to yield concentrations ranging from 10 to 1,000 μM. The samples were measured by liquid scintillation counting

(1900 CA, Tri-Carb liquid scintillation analyzer, Packard Instruments Co., Meriden, CT, USA). The unbound fraction (f_u) of PEITC was calculated as the ratio of free concentration and the total concentration.

Pharmacokinetic Study of Phenethyl Isothiocyanate

Male Sprague–Dawley rats weighing 230–260 g were purchased from Harlan Sprague–Dawley and acclimated to their surroundings for about 1 week with food and water provided *ad libitum*. The research protocol was approved by the Institutional Animal Care and Use Committee at the University at Buffalo. Two days prior to the study day, rats were anesthetized by an intramuscular injection of 90 mg/kg ketamine (Henry Schein, Melville, NY, USA) and 10 mg/kg xylazine (Henry Schein) and then implanted with cannulas (Micro-Renathane®, type MRE-040, 0.040 O.D. × 0.025 I.D., Braintree Scientific, Inc., Braintree, MA, USA) in the jugular vein. The cannula was flushed with saline containing 50 IU/mL heparin daily until the study day. Rats were fasted overnight, and each dosing group consisted of three or four animals. Phenethyl isothiocyanate was prepared in 15% hydroxypropyl-β-cyclodextrin under vigorous stirring at 50°C followed by sonication for 5 min. Intravenous doses were administered via the jugular vein cannula at doses of 2, 10, 100, or 400 μmol/kg, and saline containing 50 IU/mL heparin was administrated immediately after drug administration to completely wash the drug remaining in the cannula into the circulation. Oral doses were given by gavage at doses of 10 and 100 μmol/kg. Water was provided at all times during the study, and food was not available until 12 h after beginning the study. Blood samples (150 μL per sample) were collected into heparinized polypropylene tubes via the jugular vein cannula at 0, 5, 15, and 30 min and 1, 2, 3, 6, 9, 12, 24, 36, 48, 72, and 96 h following PEITC administration. After centrifugation at 1,000 × *g* for 6 min, the plasma was transferred into polyethylene tubes and kept frozen at −80°C until analysis.

Liquid Chromatography–Tandem Mass Spectrometry Analysis

The sample extraction and LC/MS/MS analysis of PEITC in rat plasma were performed according to the previously described procedure with minor modifications (25). Briefly, an aliquot of 100 μL of original or diluted rat plasma was transferred into a 3-mL glass tube and 1,1,2,2-$^2H_4$-PEITC was added as the internal standard. Two extractions were performed using 200 μL of *n*-hexane. The hexane extracts were combined, and 1 mL of ammonia (2 M in 2-propanol) was added to derivatize PEITC to phenethylthiourea. The mixture was then dried under a N_2 stream and reconstituted with acetonitrile/H_2O (3:2, v/v). The reconstituted sample was analyzed by LC/MS/MS using a PE SCIEX API 3000 triple–quadruple tandem mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a heated nebulizer interface, a series 2000 Perkin-Elmer pump, and a series 2000 Perkin-Elmer autosampler (Shelton, CT, USA). High-performance liquid chromatography separation was performed on a C_{18} (particle size 5 μm; 150 × 4.6 mm) column (Alltech, Deerfield, IL, USA) with a mobile phase consisted of acetonitrile/5 mM formic acid (60:40, v/v). The mass spectrometer was operated in a positive ionization mode, and multiple reaction monitoring (MRM) of MS/MS was used for specific detection of the derivatives of PEITC and the internal standard. The assay recovery of PEITC in rat plasma ranged from 89.5 ± 3.1 to 98.3 ± 6.2% for the concentrations from 50 to 1,500 nM. The quantitation limit was 4 nM, and the intra- and interday coefficients of variation were less than 5 and 10%, respectively.

Pharmacokinetic Analysis

The plasma concentration data were analyzed by noncompartmental and compartmental analyses using WinNonlin Professional Edition Version 2.1 (Pharsight, Mountain View, CA, USA) and ADAPT II (Biomedical Simulations Resource, University of Southern California, Los Angeles, CA, USA), respectively. For noncompartmental analysis, the area under the plasma concentration *vs.* time curve (AUC) was determined using the log-linear trapezoidal rule with extrapolation to infinite time. The elimination half-life ($t_{1/2}$) was estimated from the elimination rate constant (k) using the equation $t_{1/2} = \ln 2/k$, where k was determined from the terminal slope of the plasma concentration *vs.* time curve. Clearance (Cl) was calculated by dividing the dose by AUC. The maximal plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were determined directly from the plasma concentration-time curve. Volume of distribution (V_{ss}) was determined by V_{ss} = Cl(AUMC/AUC), where AUMC is the area under the first moment curve ($C \cdot t$ *vs.* t curve). Oral bioavailability (F) was determined by the ratio of the dose-normalized AUCs following oral and i.v. administration.

For the compartmental analysis, all i.v. and oral plasma concentration data were fitted simultaneously. The initial estimates for model parameters were estimated based on the values obtained from noncompartmental analysis. The appropriate model was selected using the criteria of goodness-of-fit including visual inspection, Akaike's Information Criterion, Schwarz Criterion, and correlation coefficient (r^2). Primary parameters derived from the model included first-order absorption rate constant (k_a), volume of distribution in the central compartment (V_1), Michaelis–Menten constant and maximum velocity characterizing elimination (K_{m1} and V_{max1}), volume of distribution in peripheral compartments (V_2 and V_3), peripheral clearance (Cl_{d2} and Cl_{d3}), and Michaelis–Menten constant and maximum velocity characterizing peripheral distribution (K_{m3} and V_{max3}).

Statistical Analysis

Statistical analysis was conducted using a one-way ANOVA followed by Dunnett's test or Bonferroni's test, or using a Student's t test.

RESULTS

Stability of Phenethyl Isothiocyanate

The stability data of PEITC in buffers at various pH values are summarized in Table I. Phenethyl isothiocyanate was found to degrade in the buffer solutions by first-order

Table I. Stability of Phenethyl Isothiocyanate (PEITC) in Universal Buffers at Different pH Values and Temperatures

pH	k_d (h^{-1})	$t_{1/2,d}$ (h)	Temperature (°C)
7.4	0.0125 ± 0.0013	56.1 ± 6.0	25
3.0	0.0104 ± 0.0015	68.2 ± 9.8*	25
5.0	0.0111 ± 0.0018	64.3 ± 10.6	25
8.4	0.0111 ± 0.000004	62.3 ± 0.02	25
9.3	0.0205 ± 0.0005	33.8 ± 0.8**	25
10.1	0.0462 ± 0.0067	15.3 ± 2.2**	25
7.4	0.0064 ± 0.0003	108.1 ± 4.3**	4

All experiments were performed at RT (25°C) or 4°C. The degradation half-life ($t_{1/2,d}$) was calculated from the slope of the PEITC degradation plot [log (% remaining) *vs.* pH]. One-way ANOVA followed by Dunnett's test was used to compare $t_{1/2,d}$ at different experimental conditions to $t_{1/2,d}$ at pH 7.4 under RT. Data are expressed as mean ± SE, $n = 6$ (RT), $n = 3$ (4°C).
*$p < 0.05$.
**$p < 0.001$.

kinetics. When added to a buffer of pH 7.4, PEITC was more stable than that at pH 9.3 or 10.1, with degradation half-lives ($t_{1/2,d}$) of 56.1, 33.8, and 15.3 h, respectively ($p < 0.001$). On the other hand, PEITC had a longer $t_{1/2,d}$ at pH 3 (68.2 h) than at pH 7.4 ($p < 0.05$). At pH 7.4, when stored at a refrigerated temperature (4°C), the $t_{1/2,d}$ of PEITC significantly increased to 108 h ($p < 0.001$) (Table I).

Protein Binding of Phenethyl Isothiocyanate

The optimal equilibration time for PEITC in the equilibrium dialysis experiments was 2 h. As can be seen in Table II, PEITC was highly protein bound, and the unbound fraction of PEITC in rat serum ranged from 0.0147 ± 0.0005 to 0.0227 ± 0.0005 over the concentration range of 10–1,000 μM. The average serum unbound fraction value of PEITC was 0.019. Ultrafiltration studies could not be performed because the binding of PEITC to Centrifree membranes was high; about 86% of the added PEITC was bound to the membrane.

Pharmacokinetics Studies with Intravenous Phenethyl Isothiocyanate Administration

The plasma concentration *vs.* time profiles of PEITC following its i.v. administration are shown in Fig. 2. At the lowest dose level of 2 μmol/kg, AUC, Cl, V_{ss}, and $t_{1/2}$ values were 2.96 ± 0.78 μM h, 0.70 ± 0.17 L h^{-1} kg^{-1}, 1.94 ± 0.42 L/kg, and 3.52 ± 0.35 h, respectively (Table III). When the dose was increased to 10, 100, and 400 μmol/kg, the AUC increased in a greater than proportional manner to 17.3 ± 9.3, 322 ± 149, and 807 ± 66.9 μM h, respectively (Table III). The $t_{1/2}$ at the medium dose level (100 μmol/kg) was significantly longer than that at the dose of 2 μmol/kg ($p < 0.05$), and the $t_{1/2}$ at the highest dose level (400 μmol/kg) was significantly longer than that at doses of 2 ($p < 0.001$) or 10 μmol/kg ($p < 0.01$) (Table III). The $t_{1/2}$ obtained at the 2-μmol/kg dose is shown in parenthesis in Table III because the value may be underestimated due to the fact that samples at time points later than 24 h had concentrations lower than the quantitation limit of the assay. Nonetheless, the slower elimination at higher doses suggested the involvement of nonlinear processes. Cl values were 0.70 ± 0.17 and 0.68 ± 0.29 L h^{-1} kg^{-1} at doses of 2 and 10 μmol/kg and decreased to 0.36 ± 0.18 L h^{-1} kg^{-1} at the medium dose (100 μmol/kg) and 0.50 ± 0.04 L h^{-1} kg^{-1} at the high dose (400 μmol/kg) (Table III), although these changes were not statistically significant. V_{ss} remained unchanged at doses of 2, 10, and 100 μmol/kg and was 1.94 ± 0.42, 3.27 ± 2.06, and 2.66 ± 1.22 L/kg, respectively (Table III), but V_{ss} at the 400-μmol/kg dose (5.72 ± 1.10 L/kg) was significantly higher than that at 2- or 100-μmol/kg dose levels ($p < 0.05$), suggesting nonlinearity in drug distribution following the highest dose. On the other hand, V_{ss} was variable and this limited the conclusions that may be drawn from the data.

To further examine the nonlinear nature of PEITC pharmacokinetics, the ratio values of AUC and dose were calculated and the plasma concentrations were normalized by dose. For linear pharmacokinetics, the AUC/dose ratio should remain constant because of a proportional increase in AUC with dose, and the dose-normalized plasma concentration profiles of different doses should be superimposable. In this study, the ratios of AUC/dose differed, although not significantly; the values were 1.48 ± 0.39, 1.73 ± 0.93, 3.22 ±

Table II. Protein Binding of ^{14}C-PEITC in Rat Serum

PEITC concentration (μM)	f_u (%)
10	1.47 ± 0.05
50	1.65 ± 0.01
100	1.69 ± 0.04
250	1.82 ± 0.08
500	2.02 ± 0.03
750	2.27 ± 0.21
1,000	2.27 ± 0.05

Protein binding of ^{14}C-PEITC was determined using equilibrium dialysis at 37°C using Spectra/Por dialysis tubing with a molecular weight cut-off of 12,000–14,000 MW; f_u represents the unbound fraction of PEITC and was calculated as the ratio of free concentration over the total concentration. Data are expressed as mean ± SD, $n = 3$.

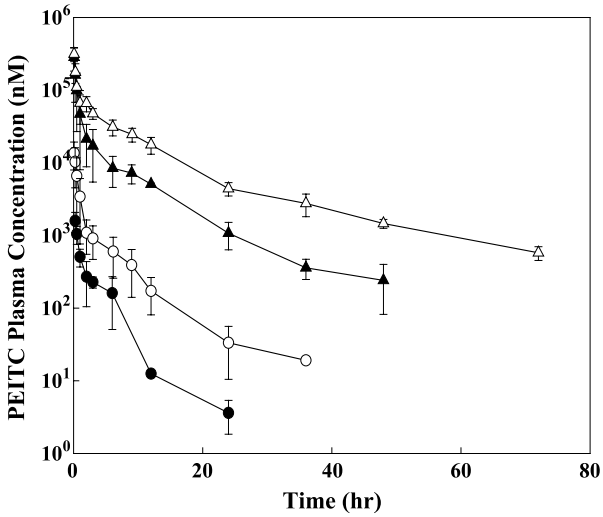

Fig. 2. The plasma concentration profile of PEITC after intravenous administration. Rats were dosed intravenously with 2 (●), 10 (○), 100 (▲), or 400 μmol/kg (△) of PEITC. Data are expressed as mean ± SD, $n = 3$ or 4.

Table III. Pharmacokinetic Parameters of PEITC after Intravenous Administration Determined by Noncompartmental Analysis

Dose (μmol/kg)	2	10	100	400
AUC (μM h)	2.96 ± 0.78	17.3 ± 9.3	322.1 ± 149.6	807.5 ± 66.9
$t_{1/2}$ (h)	(3.52 ± 0.35)	6.92 ± 3.73	9.19 ± 0.83*	13.1 ± 2.0**,***
Cl (L h^{-1} kg^{-1})	0.70 ± 0.17	0.68 ± 0.29	0.36 ± 0.18	0.50 ± 0.04
V_{ss} (L/kg)	1.94 ± 0.42	3.27 ± 2.06	2.66 ± 1.22	5.72 ± 1.10*,†
AUC/dose (kg h/L)	1.48 ± 0.39	1.73 ± 0.93	3.22 ± 1.49	2.02 ± 0.17

Noncompartmental analysis was performed by WinNonlin. Abbreviations for the parameters: AUC, area under the plasma concentration *vs.* time curve; $t_{1/2}$, elimination half-life; Cl, clearance; V_{ss}, volume of distribution. Statistics were conducted by ANOVA followed by Bonferroni's test, $n = 3$ or 4.
* $p < 0.05$ compared to the group administered with 2 μmol/kg PEITC.
** $p < 0.001$ compared to the group administered with 2 μmol/kg PEITC.
*** $p < 0.01$ compared to the group administered with 10 μmol/kg PEITC.
† $p < 0.05$ compared to the group administered with 100 μmol/kg PEITC.

1.49, and 2.02 ± 0.17 kg h L^{-1} following the doses of 2, 10, 100, and 400 μmol/kg, respectively (Table III). After normalization by dose, the plasma concentration curves did not superimpose at the four different dose levels (Fig. 3), suggesting again that the pharmacokinetics of PEITC are not linear.

Pharmacokinetic Studies with Oral Phenethyl Isothiocyanate Administration

The PEITC plasma concentration-time profiles after oral administration are shown in Fig. 4. Phenethyl isothiocyanate was rapidly absorbed and the plasma concentrations peaked at 0.44 ± 0.10 and 2.0 ± 1.0 h after doses of 10 and 100 μmol/kg, respectively (Table IV). C_{max} was 9.2 ± 0.6 and 42.1 ± 11.4 μM after doses of 10 and 100 μmol/kg, respectively. The increase of C_{max} was not proportional to the increase of dose. F decreased as the dose increased and was 114 and 93% at doses of 10 and 100 μmol/kg, respectively. The AUC values obtained after i.v. and oral administration of 10- and 100-μmol/kg doses were not significantly different, suggesting that the oral bioavailability after these two doses is close to 1.

Compartmental Modeling of Phenethyl Isothiocyanate Plasma Concentration-Time Data

Different linear and nonlinear kinetic models were used to simultaneously fit the plasma concentration data following i.v. and oral administration, including a two-compartment model, two-compartment model with Michaelis–Menten elimination, two-compartment model with Michaelis–Menten elimination and distribution, three-compartment model, three-compartment model with Michaelis–Menten elimination, and three-compartment model with Michaelis–Menten elimination and distribution. Our results showed that a three-compartment model with Michaelis–Menten functions for both the central elimination and distribution terms provided the best fitting (Fig. 5A and B). The r^2 values were 0.863, 0.625, 0.989, 0.997, 0.943, and 0.919 for the curves following oral doses of 10 and 100 μmol/kg and i.v. doses of 2, 10, 100, and 400 μmol/kg, respectively. The fitted

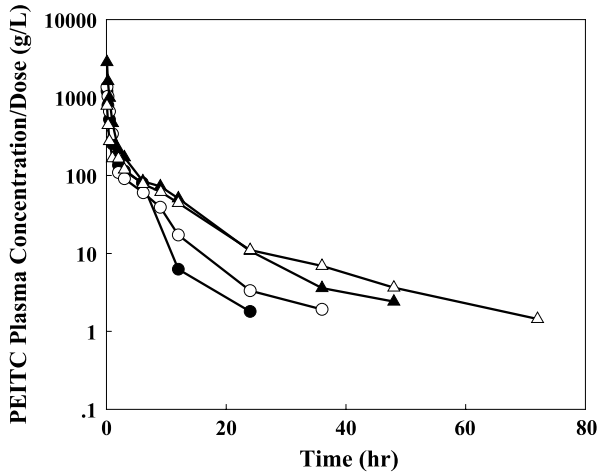

Fig. 3. The dose-normalized plasma concentration of PEITC in rats after intravenous administration. Rats were dosed intravenously with 2 (●), 10 (○), 100 (▲), or 400 μmol/kg (△) of PEITC. Data are expressed as mean ± SD, $n = 3$ or 4.

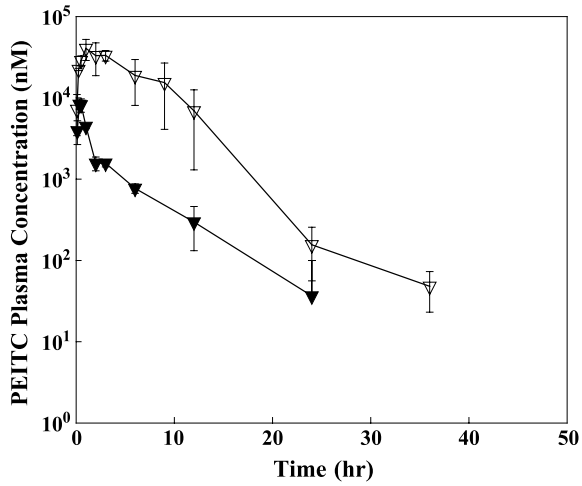

Fig. 4. The plasma concentration profile of PEITC after oral administration. Rats were dosed intravenously with 10 (▼) and 100 μmol/kg (▽) of PEITC. Data are expressed as mean ± SD, $n = 3$ or 4.

Table IV. Pharmacokinetic Parameters of PEITC after Oral Administration Determined by Noncompartmental Analysis

Dose (μmol/kg)	10	100
AUC (μM h)	19.89 ± 3.27	298.7 ± 139.4
t_{max} (h)	0.44 ± 0.10	2.0 ± 1.0
C_{max} (μM)	9.2 ± 0.6	42.1 ± 11.4
F (%)	115	93

Noncompartmental analysis was performed by WinNonlin. Abbreviations for the parameters: C_{max}, maximal plasma concentration; t_{max}, time to reach C_{max}; F, bioavailability.

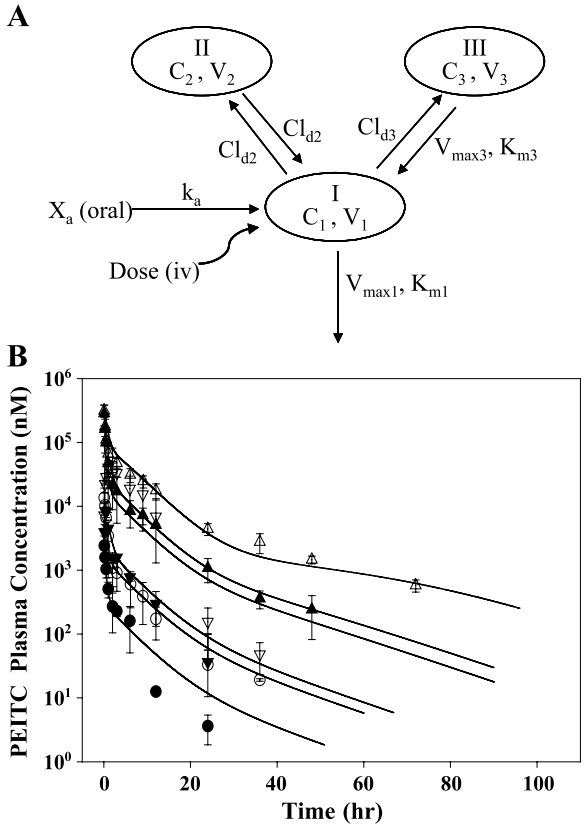

Fig. 5. The proposed compartmental model (A) and the observed and predicted plasma concentrations of PEITC (B) after intravenous and oral administration. X_a represent the drug amount at the absorption site; C_1, C_2, and C_3 represent the plasma concentrations of PEITC in central (I) and two peripheral compartments (II and III), respectively; V_1, V_2, and V_3 represent the volume of distribution of PEITC in central (I) and two peripheral compartments (II and III), respectively; k_a is the absorption rate constant; K_{m1} and V_{max1} are the Michaelis–Menten parameters to characterize central clearance from central compartment; Cl_{d2} represents peripheral clearance between peripheral compartment II and central compartment. Cl_{d3} represents peripheral clearance from central compartment to peripheral compartment III; K_{m3} and V_{max3} are the Michaelis–Menten parameters to characterize clearance from peripheral compartment III to central compartment. Rats were dosed with 2 (●), 10 (○), 100 (▲), or 400 μmol/kg (△) of PEITC intravenously, or 10 (▼) and 100 μmol/kg (▽) orally. Data are expressed as mean ± SD, $n = 3$ or 4. All the data were fitted simultaneously by ADAPT II, and the lines represent predicted plasma PEITC concentrations using the proposed compartmental model.

Table V. Estimated Pharmacokinetic Parameters of PEITC in Rats after Intravenous and Oral Administration Determined by Compartmental Analysis

Parameter	Unit	Estimated value
k_a	h^{-1}	1.8
V_1	L/kg	0.70
V_{max1}	μmol/h/kg	52.9
K_{m1}	μM	109
Cl_{d2}	L h^{-1} kg^{-1}	0.69
V_2	L/kg	1.56
Cl_{d3}	L/h/kg	0.054
V_3	L/kg	11.6
V_{max3}	μmol/h/kg	1.35
K_{m3}	μM	1.9

parameters by ADAPT II using this model are summarized in Table V.

The differential equations derived for the model (Fig. 5A) were as follows:

$$\frac{dA}{dt} = -k_a X_a \left(X_{a,0} = \text{Dose}\right) \tag{1}$$

$$\frac{dC_1}{dt} = \frac{k_a X_a}{V_1} + \frac{Cl_{d2} C_2}{V_1} + \frac{V_{max3}}{K_{m3} + C_3}\frac{C_3}{V_1} - \frac{(Cl_{d2} + Cl_{d3})C_1}{V_1} - \frac{V_{max1}}{K_{m1} + C_1}\frac{C_1}{V_1}\left(C_{1,0} = 0\right) \tag{2}$$

$$\frac{dC_1}{dt} = \frac{Cl_{d2} C_2}{V_1} + \frac{V_{max3}}{K_{m3} + C_3}\frac{C_3}{V_1} - \frac{(Cl_{d2} + Cl_{d3})C_1}{V_1} - \frac{V_{max1}}{K_{m1} + C_1}\frac{C_1}{V_1}\left(C_{1,0} = \text{Dose}\right) \tag{3}$$

$$\frac{dC_2}{dt} = \frac{Cl_{d2} C_1}{V_2} - \frac{Cl_{d2} C_2}{V_2}\left(C_{2,0} = 0\right) \tag{4}$$

$$\frac{dC_3}{dt} = \frac{Cl_{d3} C_1}{V_3} - \frac{V_{max3}}{K_{m3} + C_3}\frac{C_3}{V_3}\left(C_{3,0} = 0\right) \tag{5}$$

The initial conditions for each equation are shown in parenthesis. Equations (1), (2), (4), and (5) were used to fit the oral data and Eqs. (3), (4), and (5) were used to fit the i.v. data.

DISCUSSION

Phenethyl isothiocyanate is widely present in cruciferous vegetables as its glucosinolate precursor, gluconasturtiin. An intake of 2 oz (56.8 g) of watercress releases a minimum of ~12 mg of PEITC (16), and the amount of gluconasturtiin in Chinese cabbage is between 2 and 26 mg/100 g fresh weight (26). Hence, human consumption of PEITC through the diet is significant. More importantly, the compound possesses potent anticarcinogenic effects, and as such, PEITC is of extensive research and clinical interest. Recently, our laboratory found that PEITC could inhibit certain ATP-binding cassette transporters. However, many properties of this compound, namely, its stability, protein binding, and *in vivo* pharmacokinetics, are largely unknown.

In the present work, stability of PEITC was studied in universal buffers of citrate–phosphate–borate/HCl at pH

values of 3.0, 5.0, 7.4, 8.4, 9.3, and 10.1. All samples were studied following incubation at RT and at refrigerated temperatures. The stability of PEITC was both pH- and temperature-dependent. Phenethyl isothiocyanate degraded in a first-order manner, and the $t_{1/2,d}$ at pH 7.4 at RT was 56 h. Within the investigated pH range, PEITC is most stable at pH 3 and least stable at pH 10.1, with a $t_{1/2,d}$ decreasing from 68.2 to 15.3 h. The degradation of PEITC at pH 7.4 was significantly decreased at refrigerated temperatures, with a $t_{1/2,d}$ of 108 h at 4°C. In general, our results showed that PEITC is stable in aqueous buffers at biological pH, and that stability increased if samples were maintained at refrigerated temperatures. Our results contrasted with the report by Negrusz *et al.* (27), who indicated that PEITC is not stable at low pH in aqueous media and degrades to phenethylamine. Nonetheless, our study indicated that PEITC would be stable during sample collection and handling, provided that samples are stored under refrigerated conditions. In our pharmacokinetic study, all samples were handled immediately after collection and stored at −80°C until analysis.

In this study, we used rat serum to examine the concentration-dependent binding of PEITC. Over the concentration range of 10–1,000 μM, the binding of PEITC to serum proteins was not dependent on the compound concentration, with an average free fraction of 0.019. These results suggested that PEITC is extensively bound to protein in rat serum, likely because of the lipophilic nature of PEITC (log *P* of 3.47).

An examination of the dose-dependent pharmacokinetics of PEITC demonstrated that the clearance of PEITC decreased with increasing doses, suggesting that PEITC is eliminated in a capacity-limited manner. At doses of 2, 10, 100, and 400 μmol/kg of PEITC, $t_{1/2}$ values were 3.52 ± 0.35, 6.92 ± 3.73, 9.19 ± 0.83, and 13.1 ± 2.0 h, respectively. The $t_{1/2}$ values are smaller than the $t_{1/2}$ of 20.5 h reported in rats after the administration of a 50-μmol/kg oral dose of ^{14}C-PEITC (17). This longer $t_{1/2}$ of ^{14}C-PEITC may be a result of the slower clearance or larger volume of distribution of metabolites that contain the isotope. The major metabolic route of PEITC in rats is the mercapturic acid pathway, where PEITC undergoes glutathione conjugation followed by hydrolysis to the cysteine derivative and finally *N*-acetylation (12). At a 400-μmol/kg dose of PEITC, the clearance of PEITC was significantly slower than those of doses of 2 and 10 μmol/kg, which could be a result of saturation of glutathione *S*-transferase and/or any other enzymes involved in the mercapturic acid pathway.

The V_{ss} for PEITC ranged from 1.94 to 5.72 L/kg at doses of 2–400 μmol/kg. The high values of V_{ss} suggest that PEITC may be extensively bound to tissues. The electrophilic carbon atom of the isothiocyanate group (–N=C=S) reacts rapidly with oxygen-, sulfur-, or nitrogen-centered nucleophiles (6). It is widely accepted that ITCs covalently bind to proteins through preferentially reacting with sulfhydryl groups of amino acid residues of some proteins. Our protein binding data also indicated that PEITC is highly protein bound. Therefore, PEITC may permeate into tissues and bind to tissue proteins extensively, resulting in low unbound tissue concentrations and a high volume of distribution. The V_{ss} value at 400 μmol/kg was significantly higher than those at 100 and 2 μmol/kg. This nonlinearity could be a result of saturable active efflux from the tissues to the plasma. The glutathione conjugate of PEITC (PEITC-SG) is believed to be a substrate for MRP1 (20,28). MRP1 relies on the energy from ATP hydrolysis to efflux various anticancer drugs and toxins and is present ubiquitously in the human body and tumors including both solid tumors and hematological malignancies (29,30). At high doses, the metabolism of PEITC to PEITC-SG may be saturated or MRP1-mediated efflux may be inhibited because of depletion of intracellular glutathione (20), resulting in higher PEITC tissue concentrations and thus a higher apparent volume of distribution.

Following oral administration of PEITC, peak plasma concentrations occurred rapidly at 0.4 ± 0.1 and 2.0 ± 1.0 h following doses of 10 and 100 μmol/kg with a k_a of 1.8 h^{-1}. In our previous clinical study, PEITC has an apparent absorption rate constant (k_a) of 1.3 h^{-1} and t_{max} of 2.6 h in healthy subjects after consumption of 100-g watercress (24). The values are comparable to what we observed in rats. Taken together, PEITC exhibits rapid absorption and high availability. Conaway *et al.* (17) reported that peak whole blood ^{14}C concentrations occurred at 2.9 h in rats following a dose of 50 μmol/kg ^{14}C-PEITC. The longer t_{max} reported when measuring the ^{14}C radiolabel may reflect the contribution of hydrophilic metabolites, formed from PEITC, to the total radioactivity measured. In that study, the mean whole blood ^{14}C C_{max} value was 18.77 μM (17), which is within the range we observed for our 10- and 100-μmol/kg doses (C_{max} values of 9.2 ± 0.6 and 42.1 ± 11.4 μM, respectively).

Following doses of 10 and 100 μmol/kg, the *F* values of PEITC were 115 and 93%, respectively, and the AUCs were not significantly different for oral and i.v. administration, suggesting that PEITC was almost completely absorbed after oral administration. As a small hydrophobic compound, PEITC permeates cells easily by passive diffusion; therefore, good intestinal absorption would be expected. In addition, high oral bioavailability suggests that first-pass metabolism may be negligible. Although PEITC can undergo glutathione conjugation, this may not be extensive in the intestine and liver; alternatively, or in addition, PEITC-SG may undergo hydrolysis, releasing PEITC that can diffuse out of the intestinal cells and hepatocytes.

The compartment model presented was chosen based on goodness of fit and for its physiological relevance. Compartment I represents the central compartment, where PEITC was eliminated in a capacity-limited manner, as a result of saturable metabolism. Compartment II represents the tissues into which PEITC diffuses freely and distributes in a linear manner. Compartment III represents those tissues that express MRP1 or other forms of MRP, where there is active efflux of PEITC/PEITC-SG. Phenethyl isothiocyanate enters cells by passive diffusion; therefore, Cl_{d3}, the peripheral clearance from compartment I to III, is linear. Once PEITC enters cells, it may conjugate with glutathione rapidly. It has been reported that the intracellular accumulation of ITC-SG is maximal after about 30 min in cultured cells (31), and the intracellular ITC-SG may be further metabolized or hydrolyzed to liberate ITCs due to the reversible conjugation (32). Therefore, if not hydrolyzed back to PEITC, the formed PEITC-SG conjugate may be effluxed by MRP1 back to the central compartment. The fitted K_{m1} and K_{m3} were 109 and

1.9 μM, respectively. At low PEITC concentrations, the plasma concentration-time profile could be fit by a linear model because plasma concentrations were much lower than the two K_m values; at high concentrations, greater than the K_m, the nonlinear nature became evident. Our analysis showed that following a 2-μmol/kg dose, the plasma concentration of PEITC exhibited an apparent linear two-compartment model. When the dose was increased, however, a three-compartment model fit the plasma concentration data better. When the dose was increased 200-fold to 400 μmol/kg and plasma samples were collected up to 72 h, a three-compartment model with saturable efflux compartment best fit the plasma concentration data. Previously, the time course of whole blood radioactivity, following an oral dose of 50 μmol/kg ^{14}C-PEITC to rats, has been reported to fit a two-compartment linear model (17). This difference, from the present investigation, may be because of the fact that the ^{14}C reflects the total of PEITC and its metabolites; the metabolite disposition may be different from that of the parent. Additionally, the sampling was sparse, especially during the distribution phases, compared with the present investigation. In our study, the dose range we used was very large, and we were therefore able to observe nonlinearity in clearance for the high-dose group. Adding additional dose groups and more animals per group would help decrease the variability in the data and better elucidate the nonlinearities in the disposition of PEITC.

In summary, PEITC degrades in aqueous buffers with a half-life of 56.1 h at pH 7.4 at RT, and this degradation half-life increases to 108 h when samples are stored at 4°C. The protein binding of PEITC is high (98.1%) and is not concentration-dependent. Oral bioavailability of PEITC in rats is high and was 115 and 93% following doses of 10 and 100 μmol/kg, respectively. The clearance following a 2-μmol/kg dose of PEITC was 0.7 ± 0.17 L h^{-1} kg^{-1} and was 0.36 ± 0.18 and 0.50 ± 0.04 L h^{-1} kg^{-1} at 100- and 400-μmol/kg doses, respectively. The V_{ss} following a 2-μmol/kg dose of PEITC was 1.94 ± 0.42 L/kg and was largely unchanged following 10- and 100-μmol/kg doses (3.27 ± 2.06 and 2.66 ± 1.22 L/kg, respectively), but increased to 5.72 ± 1.10 L/kg following a dose of 400 μmol/kg. A three-compartment model with Michaelis–Menten elimination and distribution provided the best fit of the plasma concentration-time data following oral and i.v. administration in rats.

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