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Pharmacokinetic, safety and tolerability studies after single and multiple oral administration of Phenethyl isothiocyanate in Nutri Jelly

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Background : *Phenethyl isothiocyanate (PEITC) is a dietary phytochemical with anti-cancer properties. Recently, it was added to Nutri Jelly, a nutritious gel developed for cancer patients with eating and swallowing problems.*

Objectives : *To evaluate pharmacokinetics, safety and tolerability of isothiocyanates after single- and multiple-oral administrations of 40 mg of PEITC in Nutri Jelly in healthy volunteers.*

Methods : *This was an open-label, single- and multiple-dose study. Ten subjects received a single dose of 40 mg of PEITC in Nutri Jelly and continued with this dose once daily for 5 days. Serial plasma samples at various times after the administration on day 1 and 5 were collected to determine total isothiocyanate levels using HPLC-based cyclocondensation assay. Single- and multiple-dose pharmacokinetics were analyzed using non-compartmental analysis. Safety and tolerability assessments included physical examination, electrocardiogram, clinical laboratory tests and adverse events.*

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Results : *After single administration, maximum concentrations of isothiocyanates were observed at 2.65 ± 0.89 h (mean ± SD).The mean apparent volume of distribution and oral clearance were 1.13 ± 0.91 L/kg and 22.16 ± 4.31 L/h, respectively. Isothiocyanates were rapidly eliminated with the average terminal half-life of 1.75 ± 0.93 h. After multiple-dose administration, the mean accumulation index was 1.003 ± 0.007. There was no serious adverse event reported and no clinically significant abnormality was found in any of the clinical and biochemical parameters.*

Conclusions : *Nutri-PEITC Jelly was well-tolerated in healthy subjects with mild adverse events. Total isothiocyanates were rapidly eliminated with a short half-life. No significant accumulation was observed upon repeated doses for 5 days.*

Keywords : *Phenethyl isothiocyanate, PEITC, Nutri-PEITC Jelly, pharmacokinetics, single-dose, multiple-dose.*

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เหตุผลของการทำวิจัย : *ฟีเนทิลไอโซไธโอไซยาเนตเป็นสารพฤกษเคมีซึ่งมีฤทธิ์ต้านมะเร็ง ปัจจุบันมีการผสมสารดังกล่าวในเจลลี่โภชนาซึ่งเป็นอาหารสำหรับผู้ป่วยโรคมะเร็งที่มีปัญหาการกลืนและเคี้ยว*

วัตถุประสงค์ : *เพื่อศึกษาเภสัชจลนศาสตร์ความปลอดภัย และความทนทานของไอโซไธโอไซยาเนตหลังการรับประทานเจลลี่โภชนาที่มีฟีเนทิลไอโซไธโอไซยาเนตแบบครั้งเดียวและแบบหลายครั้ง*

ตัวอย่างและวิธีการศึกษา : *เป็นการศึกษาแบบไม่ปกปิด อาสาสมัครสุขภาพดี 10 คนได้รับเจลลี่โภชนาที่มีฟีเนทิลไอโซไธโอไซยาเนต 40 มิลลิกรัม วันละ 1 ครั้งเป็นเวลา 5 วัน เก็บตัวอย่างพลาสมาที่เวลาต่าง ๆ ในวันที่ 1 และ 5 เพื่อหาระดับความเข้มข้นของไอโซไธโอไซยาเนต โดย HPLC-based cyclocondensation assay วิเคราะห์ทางเภสัชจลนศาสตร์ด้วยวิธี non-compartmental analysis ประเมินความปลอดภัยและความทนทานจากการตรวจร่างกาย การตรวจทางห้องปฏิบัติการ และอาการไม่พึงประสงค์*

ผลการศึกษา : *หลังการให้แบบครั้งเดียว พบระดับไอโซไธโอไซยาเนตสูงสุดที่เวลา 2.65 ± 0.89 ชั่วโมง ค่าปริมาตรการกระจาย (Vd/F) และอัตราการกำจัด (Cl/F) เท่ากับ 1.13 ± 0.91 ลิตรต่อกิโลกรัม และ 22.16 ± 4.31 ลิตรต่อชั่วโมงตามลำดับ ไอโซไธโอไซยาเนตถูกขจัดออกจากร่างกายอย่างรวดเร็ว โดยมีค่าครึ่งชีวิตของการขจัด 1.75 ± 0.93 ชั่วโมง เมื่อให้ติดต่อกัน 5 วัน พบค่าดัชนีการสะสม 1.003 ± 0.007 ไม่พบความผิดปกติจากการตรวจร่างกายและผลทางห้องปฏิบัติการตลอดจนไม่พบอาการไม่พึงประสงค์ที่รุนแรง*

สรุป : *การให้เจลลี่โภชนาที่มีฟีเนทิลไอโซไธโอไซยาเนตแก่อาสาสมัครสุขภาพดีมีความปลอดภัย ไม่พบการสะสมของไอโซไธโอไซยาเนตที่มีนัยสำคัญหลังได้รับติดต่อกัน 5 วัน*

คำสำคัญ : *ฟีเนทิลไอโซไธโอไซยาเนต, เภสัชจลนศาสตร์, เจลลี่โภชนา, แบบครั้งเดียว, แบบหลายครั้ง.*

Cancer is the leading cause of deaths worldwide, accounting for 8.2 million deaths in 2012 and predicted to rise to 13 million within the next two decades.[1] Oral cancer, the 15th most common cancer, often diagnosed in advanced stage, is difficult to treat.[2, 3] The disease and the side effects of its treatment may affect patients' ability to eat and lead to malnutrition and poor quality of life in patients.[4] These side effects include pain, weakness, altered facial appearance, dry mouth, and difficulty in swallowing or chewing food.[5] The Dental Innovation Foundation under the Royal Patronage(DIF) has developed and formulated the food gel with semisolid texture called "Nutri Jelly" which is a ready-to-eat nutritious gel with 230 – 260 kcal per serving (1 kcal/ 1 ml). This product has been shown to support the nutrition and improve health related quality of life (HRQOL).[6, 7] To enhance its benefits, Nutri Jelly was recently modified by adding an anticancer agent, phenethyl isothiocyanate (PEITC).

PEITC is a metabolite of glucosinolates which is naturally present in cruciferous vegetables such as watercress, broccoli, wasabi and cabbage.[8, 9] PEITC is a low molecular weight (MW = 163.24 g/mole) compound and is lipophilic (log P = 3.47).[10] *In vitro* and *in vivo* studies have indicated that PEITC has anticancer effects involving a number of distinct mechanisms and is more selective to the cancer cells than the normal cells.[11-14] In animal model of cancer, PEITC has been reported to reduce tumor volume and prolong survival time of nude mice.[13, 15] However, the anticancer effect of PEITC in human has not been reported. PEITC is mainly eliminated by metabolism in humans and animals.[14, 16, 17] The metabolites, mercapturic acid and PEITC-N-acetyl cysteine are excreted in the urine.[18, 19]

In 2001, Liebes *et al*. developed a HPLC based cyclocondensation assay to determine total isothiocyanate levels in human plasma.[20] This assay does not require radiolabelling unlike the earlier methods [21, 22] but it could not be distinguished from its metabolites. They also reported pharmacokinetics of isothiocyanates after three healthy subjects having taken single dose of 40 mg PEITC in olive oil. A one-compartment pharmacokinetic model was used to describe the data. The pharmacokinetic parameters were reported in mean ± SE as follows: the peak plasma concentration (C_{max}) 1.04 ± 0.22 μM, the time to reach C_{max} (T_{max}) 4.6 ± 0.7 h, oral clearance (CL/F) 236.00 ± 36.80 mL/m^2/min, and half-life 3.70 ± 1.30 h. In 2003, Ji and Morris developed a LC-MS/ MS method that could determine unchanged PEITC but this analytical approach that involves ammonia derivatization for 6 hours and sophisticated instrument.[9] They also reported that one compartment model best described the pharmacokinetic of PEITC after four healthy subjects having taken 100g of watercress (equivalent to 25 mg of PEITC) and the pharmacokinetic parameters were reported in mean ± SD as follows: C_{max} 928.5 ± 250 nM, T_{max} 2.6 ± 1.1 h, CL/F 29.5 ± 10.8 L/h, apparent volume of distribution (Vd/F) 154.5 ± 46.8 Land half-life 4.9 ± 1.1h. However, these findings were from limited number of subjects receiving only single dose administration. Currently, no pharmacokinetic profile after multiple dose administration of PEITC has been reported.

To combine anti-cancer effects of PEITC and provide nutritional support for cancer patients, Nutri-PEITC Jelly was recently developed by adding PEITC as a supplement into Nutri Jelly during gel

polymerization.[6] The product was tested in animal model for acute and sub-acute toxicity according to the guideline of the Organization for Economic Cooperation and Development (OECD).[23] Currently, there is no direct phase I study to assess adverse events and clinical laboratory effects of Nutri-PEITC Jelly in humans. In this study, we aimed to determine safety and tolerability after a single and multiple doses of oral dose administration of 40 mg of PEITC in Nutri Jelly in healthy volunteers and also to investigate pharmacokinetics of isothiocyanates after Nutri-PEITC administration.

Material and Methods

This study was performed in accordance with the principles of Good Clinical Practice (GCP). The final protocol has been approved by the Ethics Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University (protocol review no. 13-33-023). All subjects were informed about the potential risks in participating in the study and they provided written informed consents before enrollment.

Subjects

To be eligible for this trial, male and female subjects must fulfill the following criteria: 18 - 55 years of age; BMI 18-23 kg/m^2; non-smoker; living in a healthy condition, as evaluated by physical examinations, clinical laboratory tests (complete blood count, lipid profile, fasting blood sugar, liver and renal function tests), vital signs and electrocardiography (12-lead ECG). Subjects were excluded if they had any history of chronic diseases such as renal, hepatic, respiratory, cardiovascular and gastrointestinal disorder. Female participants were excluded if they were pregnant or breast feeding.

All subjects were abstained from consuming acetaminophen, chlorxozaxone and alcoholic beverage 24 hours before and throughout the study because their metabolic pathways may interfere with PEITC metabolism.[24, 25] They were required to refrain from any product containing PEITC or other isothiocyanates including watercress, broccoli, Chinese broccoli, beetroot, cabbage, radish, coriander, onion, shallot and wasabi for 72 hours before and throughout the study.

Preparation of Nutri-PEITC Jelly

Nutri-PEITC Jelly was formulated and manufactured by Dental Innovation Foundation under Royal Patronage. Each box of 200 g Nutri-PEITC Jelly contained 20 mg PEITC (0.01% w/w) and was stored at room temperature. All subjects were required to take two boxes of Nutri-PEITC Jelly (40 mg of PEITC).

The dose of 40 mg of PEITC was selected based on a human equivalent dose of 0.81 mg/kg (Dental Innovation Foundation under Royal Patronage research report; unpublished data) assuming the average weight for a Thai cancer patient as 50 kg. This dose (0.81 mg/kg) is actually much lower than one-tenth of the lethal dose to 10% of mice (LD10) which could be used as a safe human starting dose according to the US Food and Drug Administration's guidance.[26] In addition, previously a single dose of 40 mg PEITC was safely given orally to human subjects.[20]

Study Design

This was an open-label, single- and multiple-dose study. All participants received a single dose of 40 mg PEITC (two boxes of 200 g Nutri-PEITC Jelly)

in the morning of day 1 after an overnight fast (at least 8 hours) and then continued with the same dose once daily for 5 days for multiple-dose study. The elimination half-life of isothiocyanates in previous studies ranged from 1.7 - 6.2 hours [9, 20], but there was no data with repeated dosing prior to the present study. To be certain that a steady state was reached, we assumed that the period of 5 days should be sufficient. Subjects were required to stay at the study center for at least approximately 14 h on day 1 and day 5 for blood sample collection. Standard meals and drinking water were provided at the center at 1, 5 and 10 hours after dosing. Beverages were allowed after 2 hours from PEITC intake. Subjects were instructed to record daily dietary intake in a provided food diary throughout the study.

Blood Sampling

As for pharmacokinetic analysis, serial blood samples (5 mL at each time point) were collected into sodium heparin-containing tubes by an indwelling catheter inserted into the forearm at 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12 and 24 hours after dosing. Within 2 hours of each collection, the blood samples were centrifuged 1,000g at 4 ^{o}C for 10 minutes and their supernatants (plasma) were collected. Plasma samples were stored at -20 ^{o}C until analysis.

Analytical methods

The sample extraction and quantification of isothiocyanates in plasma were performed by cyclocondensation, followed by a two-step hexane extraction and analyzed by HPLC-UV at 365 nm according to the previously described procedure with minor modifications.[20] After double extraction by 2 mL of n-hexane, the supernatant was collected and transferred to 10-mL glass test tube. The combined extracts were evaporated to dryness using Centrivap® (Labconco, Fort Scott, KS) at room temperature. The remaining residue was reconstituted with 0.5 mL methanol: water (70:30,v/v) and mixed by vortex for 1 minute. The obtained samples were transferred to 2.0 mL micro-centrifuged-tube and centrifuged as the prior method indicated. The injection volume of sample was 20 μL. Concentrations of isothiocyanates in each sample were calculated from the chromatogram peak area of 1,3-benzenedithiol-2-thionein each chromatogram, using linear regression analysis of standard curve.

Prior to analysis of study samples, the method was validated to assure reliability of the performance. The lower limit of quantitation (LLOQ) was 16.32 μg/L with the coefficient of variation (CV) of 16.97 %. The linear calibration curve was obtained over the concentration range of 16.32 - 652.96 μg/L. The intra-day accuracy of low, medium and high quality control (32.64, 130.59, 261.18 μg/L, respectively) ranged from 88.79 - 102.56% and the inter-day accuracy ranged from 90.01 - 97.58%. As for the precision, %CV values were 1.90 - 14.97% for intra-day analysis and 2.78 - 10.00 % for inter-day analysis.

Safety and tolerability evaluation

Safety and tolerability were evaluated throughout the study. Complete physical examination, vital signs, 12-lead electrocardiograms, and clinical laboratory measurements (blood chemistry, hematology) were performed at baseline and at 24 hours after the last repeated dose. Adverse events (AEs) were identified through spontaneous reports,

subject interview and clinical evaluation. AEs were assessed by the investigator as to their severity, duration and relationship to the study treatment.

Pharmacokinetic and statistical analysis

Non-compartmental analysis was performed using Phoenix WinNonlin version 6.3 (Certara USA Inc, St. Louis, MO).The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined directly from the observed data. The elimination rate constant (λ) was determined by linear regression. The area under the plasma concentration-time curve (AUC) from time zero to t (AUC_{0-t}), where t or T_{last} is the time of last measured concentration, was calculated by using the linear trapezoidal rule. The AUC from time zero to infinity (AUC_{0-INF}) was calculated as: $AUC_{0-t} + (C_{last}/\lambda)$, where C_{last} is last measured concentration and λ is the slope of the terminal phase. The elimination half-life ($T_{1/2}$) was calculated as $\ln 2/\lambda$. The oral clearance (CL/F) was estimated as the dose divided by AUC_{0-INF} and the apparent volume of distribution (Vd/F) was determined as CL/F divided by λ. For multiple-dose analysis, the accumulation index was calculated as:$1/(1 - e^{-\lambda\tau})$, where τ is dosage interval.

Statistical analysis was performed using SPSS Statistic for Windows, Version 17.0. (SPSS Inc., Chicago, IL). The results were expressed as the mean ± SD. The difference of pharmacokinetic parameters in single- and multiple-dose was analyzed by Wilcoxon signed-rank test. A *P*-value of less than 0.05 was considered statistically significant.

Results

Subject demographics

A total of 10 healthy subjects (5 males and 5 females) were enrolled in the study. The mean ± SD age of the subjects was 31.9 ± 8.2 years. Mean weight and height of the study subjects were 53.7 ± 5.9 kg and 163 ± 6 cm, respectively, with a calculated mean BMI of 20.12 ± 1.35 kg/m^2. All 10 subjects completed the trial.

Safety and Tolerability

Throughout the single- and multiple-dose periods, no clinical significant changes in hematology and blood chemistry test results were observed including complete blood count with differential and platelet count, blood urea nitrogen, serum creatinine, liver enzyme, total bilirubin, direct bilirubin, alkaline phosphatase, serum albumin level, total cholesterol, triglycerides, and fasting blood sugar.

Vital sign values (blood pressure and pulse) and 12-lead electrocardiograms were within the normal range. There was no subject discontinued from the study due to an adverse event (AE). A total of 5 mild AEs were reported and considered Nutri-PEITC Jelly related, including mild diarrhea on day 1 (30%), flatulence (10%) and pruritus (10%). All subjects that had AEs did not require any treatment.

Pharmacokinetics

Although all subjects were required to refrain from cruciferous vegetables for at least 3 days, prior to and throughout the study, isothiocyanates were detected in all 10 subjects at time 0 (T_0) before Nutri-PEITC Jelly administration. The mean background isothiocyanate levels in plasma at T_0 on day 1 and day 5 were 61.76 ± 18.59 (range 30.89 - 93.25) and 110.59 ± 62.82 (range 17.01 - 210.40) μg/L,

respectively. There was no statistically significant difference in the concentrations at T_0 between day 1 and day 5 (P = 0.059). Based on the results of this study that the elimination half-life of isothiocyanates from single-dose data was 1.75 ± 0.93 h (as shown in Table 1) and the time of last measured concentration (T_{last}) ranged from 6 to 12 h, each subject's background isothiocyanate concentration at T_0 on either day 1 or day 5 was subtracted from each subsequent isothiocyanate concentration. Pharmacokinetic analysis was performed on the baseline-corrected values. Any negative values after baseline correction were treated as zero.

The pharmacokinetic parameters of isothiocyanates after single and multiple administration of Nutri-PEITC Jelly are summarized in Table 1. Isothiocyanates were absorbed with a mean T_{max} of 2.65 ± 0.89 h after single-dose administration which was relatively identical to the multiple-dose (day 5) value of 2.66 ± 1.17 h. After both single- and multiple-dosing, the concentrations of isothiocyanates returned to baseline levels within 12 h (T_{last} ranging from 6 to 12 h). Almost all pharmacokinetic parameters showed no significant difference between single and multiple dosing (P >0.05). Only C_{max} showed a significant increase from 357.36 ± 94.82 μg/L after single administration to 491.89 ± 76.38 μg/L after multiple administration (P <0.05). AUC tended to be higher, but the difference was not significant. Under steady-state conditions (day 5), isothiocyanates were rapidly cleared up from plasma with a half-life of 1.88 ± 1.19 h, which was not statistically different from the single dose (day 1) value of 1.75 ± 0.93 h. The accumulation index was 1.003 ± 0.007. Figure 1 shows plasma isothiocyanates concentration-time curve after single- and multiple-administration of Nutri-PEITC Jelly.

Table 1. Pharmacokinetic parameters of isothiocyanates after single and multiple oral administration of Nutri-PEITC Jelly.

Pharmacokinetic Parameters	**Single dose (mean ± SD)**	**Multiple dose (mean ± SD)**
C_{max} (μg/L)	357.36 ± 94.82	491.89 ± 76.38*
T_{max} (h)	2.65 ± 0.89	2.66 ± 1.17
T_{last} (h)	10.22 ± 2.37	9.00 ± 2.71
$AUC_{0\text{-}last}$ (μg/L • h)	1,755.07 ± 546.83	2,434.57 ± 620.35
$AUC_{0\text{-}INF}$ (μg/L • h)	1,885.69 ± 474.23	NA
$T_{1/2}$ (h)	1.75 ± 0.93	1.88 ± 1.19
Vd/F (L)	56.88 ± 38.75	41.89 ± 19.31
Vd/F (L/kg)	1.13 ± 0.91	0.78 ± 0.35
CL/F (L/h)	22.16 ± 4.31	16.84 ± 4.34
Accumulation Index	NA	1.003 ± 0.007

* p-value < 0.05, Wilcoxon signed-rank test

NA = Not Available

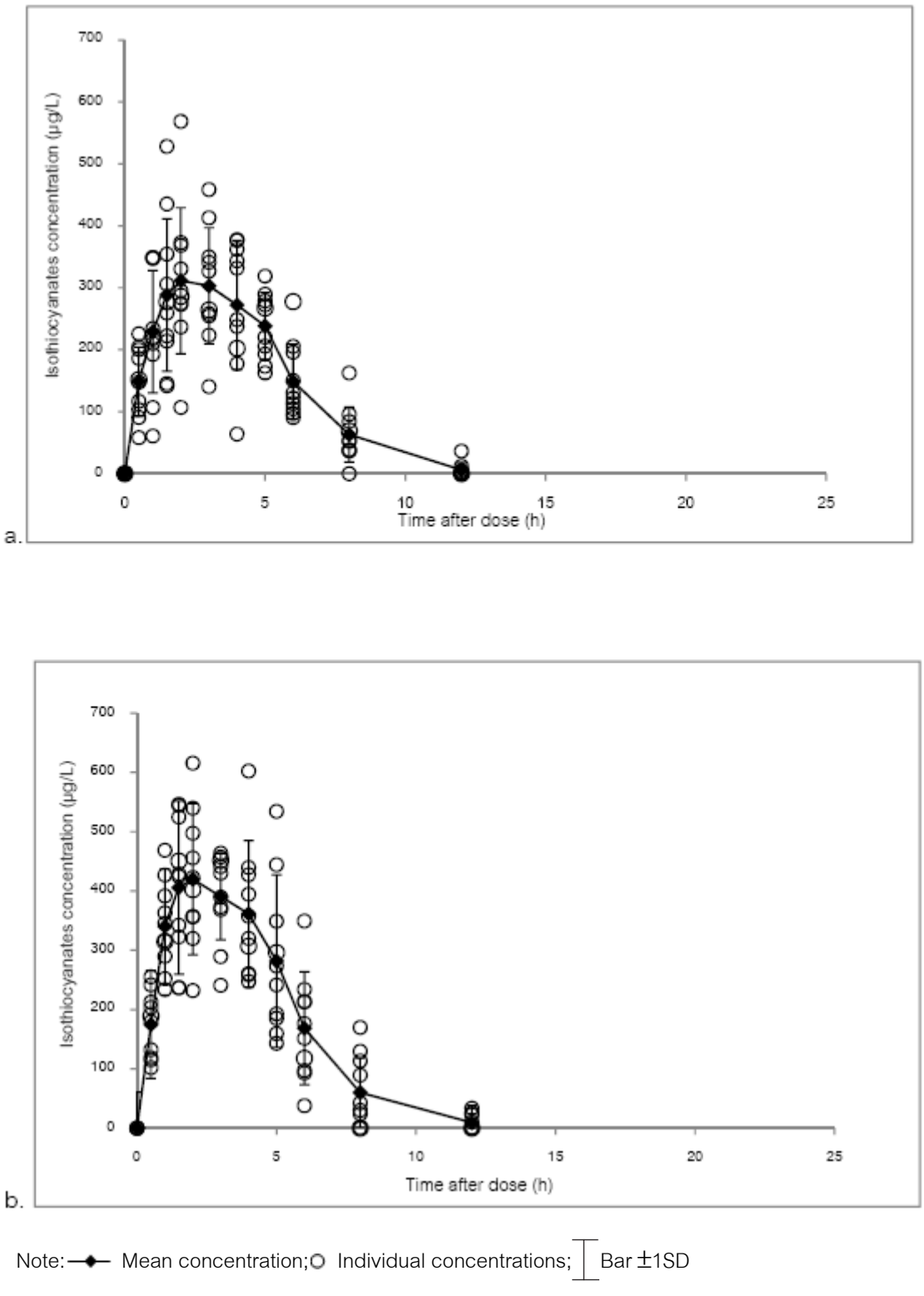

Figure 1. Plasma isothiocyanates concentration-time curve in healthy Thai volunteers (n = 10) a. single dose (day 1) b. multiple doses (day 5).

Discussion

Phenethyl isothiocyanate (PEITC) naturally appears as isothiocyanate with chemopreventive and chemotherapeutic activities.[11 - 15] Animal studies showed that PEITC can prolong the survival time of tumor-implanted animals.[13] Because of its activity, the Dental Innovation Foundation under the Royal Patronage (DIF) has formulated that PEITC in Nutri Jelly could be a novel palliative care for cancer treatment. Here, we first reported pharmacokinetic behaviors of isothiocyanates, safety and tolerability after single and multiple administration of 40 mg of PEITC in Nutri Jelly.

Nutri-PEITC Jelly did not cause any clinically significant abnormalities or changes in routine clinical laboratory and 12-lead ECG in all subjects. The reported AEs were pruritus, diarrhea and flatulence. The AEs involving gastrointestinal system could happen due to the ingredients in Nutri Jelly, such as agar and gelatin, which could increase gastrointestinal osmotic pressure.[6] The reported AEs were mild and disappeared when discontinued the product without any treatment. These results suggest that Nutri-PEITC Jelly is safe and that its AEs are generally mild and tolerable.

Ten subjects (male: female = 1:1) completed the study. We used the HPLC based cyclocondenstation assay developed by Liebes *et al.*[20] to measure total isothiocyanate levels in plasma samples. Although we provided all subjects with a list of restricted foods known to contain PEITC and other isothiocyanates, we still found the plasma isothiocyanates concentration at baseline of 61.76 ± 18.59 μg/L in the single-dose phase, which was very close to that of Liebes *et al.* (56.48 ± 27.42 μg/L).[20] Although Ji and Morris developed the LC-MS/MS assay to specifically determine unchanged PEITC, they also found low concentrations of PEITC in all subjects before the initiation of the PEITC study but after 3 days of dietary restriction. These findings suggest that there might be other unknown dietary sources of PEITC and other isothiocyanates. Additional dietary sources of PEITC and background of PEITC level in Thai subjects should be further explored.

The pharmacokinetic parameters from our study were compared with that of Liebes *et al.*[20], which used the same dose of 40 mg PEITC but formulated in oil solution. Both studies used the HPLC based cyclocondenstation assay. Isothiocyanates from Nutri-PEITC Jelly was more rapidly absorbed than that of PEITC in oil (T_{max} of 2.6 h, compared to 4.6 h). The mean C_{max} was found to be 357.36 μg/L and the mean AUC was 1,885.69 μg/L • h, indicating significantly higher bioavailability compared with the values of Liebes' study (C_{max} 169.77 μg/L and AUC 1714.02 μg/L • h). These findings suggest that Nutri Jelly formulation may accelerate the absorption of PEITC. It is likely that certain ingredients in Nutri Jelly might enhance gastric emptying and increase the diffusion of PEITC through the intestinal membrane. The underlying mechanism should be further explored. It is worth noting that the ethnic background of subjects in our study is different from that of Liebes *et al.*[20]Patel *et al.*[27] have studied the effect of ethnic differences on metabolism of acetaminophen, which partly metabolized via mercaptopyruvic pathway similar to PEITC. They found that the fractional excretion of cysteine and mercapturate conjugates in the Caucasian was significantly higher than that in

the Oriental. However, the CL/F of isothiocyanates in our study (10 Asians, 22.16 L/h) was not significantly lower than that in the study of Liebes *et al*. (3 Caucasians, 24.49 L/h).[20]

In multiple-dose phase, the mean ± SD isothiocyanates concentration at T_0 on day 5 was 110.59 ± 62.82 μg/L. It appeared to be higher than baseline concentration in single-dose study but not statistically significant (P >0.05). The C_{max} was significantly increased following multiple dosing but other pharmacokinetic parameters were comparable. The difference in C_{max} could be due to the large variability of background isothiocyanates levels and small sample size. Nonetheless isothiocyanates levels were quickly back to the baseline within 12 hours after dosing. The elimination half-life of isothiocyanates (~2 h) is relatively short compared with the dosing interval (24 h) and the mean accumulation index was 1.003, indicating that most of isothiocyanates was eliminated between doses with very little accumulation.

A limitation of this study was that there was no placebo group. This is due to the fact that PEITC has wasabi-like pungent flavor which makes it difficult to produce placebo with similar smell and taste. Therefore, there is doubt whether the AEs involving gastrointestinal system were from PEITC or other ingredients of Nutri Jelly.

Conclusion

This study demonstrated that 40 mg of PEITC in Nutri Jelly administered orally to healthy volunteers was safe and well tolerated. This study shows single- and multiple-dose pharmacokinetic parameters. No significant accumulation was observed with repeated PEITC in Nutri Jelly for 5 days.

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