Original Article

Divergent Effects of Turmeric Crude Extract on P-Glycoprotein Activity in Healthy Male Subjects: A Randomized Crossover Study

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Background: Curcuminoids, major ingredients of turmeric crude extract, have shown P-glycoprotein [P-gp] inhibitory properties in both in vitro and in vivo studies. However, their potential interaction with drugs known as P-gp substrates in humans is not characterized.

Objective: To investigate the effect of turmeric crude extract on P-gp activity in healthy subjects.

Materials and Methods: This preliminary, open-label, randomized, two-period, crossover study was conducted in 12 healthy male volunteers. Pharmacokinetic [PK] parameters of digoxin, a P-gp probe, were measured following a single oral dose of 0.5 mg digoxin administered without and with concurrent use of turmeric extract (equivalent to 500 mg of curcuminoids, given twice daily for four days prior and three days after a single dose of digoxin).

Results: Considering ±25% change of maximum plasma concentration [C_{max}] of digoxin in each individual subject to be of clinical relevance, three categories of interaction between turmeric extract and digoxin were observed. With concurrent use of turmeric extract, C_{max} of digoxin increased, remained unchanged, and decreased in six, four, and two subjects, respectively.

Conclusion: These findings suggest that medical professionals should be aware of possible divergent interactions between turmeric crude extract and P-gp substrates for the proper monitoring of their individual patients.

Keywords: Turmeric extract, Digoxin, Pharmacokinetics, Drug interactions, P-glycoprotein, Healthy volunteers

J Med Assoc Thai 2018; 101 (3): 313-21
Website: http://www.jmatonline.com

P-glycoprotein [P-gp], also known as multidrug resistant protein 1 [MDR1], is an ATP-dependent efflux transporter found in the intestines, kidneys, liver, and the blood-brain and blood-testis barriers, as well as in the placenta[1]. It is involved in drug disposition as a protective mechanism of the body against xenobiotic compounds, excreting these compounds into the intestinal lumen, urine, and bile, and preventing their accumulation in vital organs such as the brain, testes, and placenta[2]. Many drugs that are identified as P-gp substrates may interact with a P-gp inhibitor or a P-gp inducer, resulting in toxicity or loss of efficacy of such substrates.

Digoxin, a purified cardiac glycoside used to treat various heart conditions, is usually applied as an in vivo P-gp probe in clinical pharmacokinetic [PK] drug-drug interaction [DDI] studies[3,4]. It is selective for and mainly mediated by P-gp transporters, as well as it could be administered safely in human DDI studies[4,5]. Concurrent use of a drug known as a P-gp inhibitor (e.g., quinidine) with digoxin can lead to an increase in plasma digoxin concentration in the body, whereas concomitant use of digoxin with a P-gp inducer (e.g., rifampin) can cause a decrease in digoxin plasma concentration[6-8].

Turmeric (*Curcuma longa* L.) is a perennial herb found throughout tropical and subtropical regions. Major biologically active compounds in turmeric crude extract are curcuminoids, which typically include curcumin, demethoxycurcumin, and bisdemethoxycurcumin[9]. Non-clinical evidence has demonstrated the P-gp inhibitory property of curcuminoids in a variety of human cell lines and animal experiments[10-13]. The inhibition of P-gp-mediated digoxin (a P-gp probe) transport by curcumin from turmeric extract has been shown to

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How to cite this article: Koonrungsesomboon N, Hanprasertpong N, Potikanond S, Karbwang J, Teekachunhatean S. Divergent effects of turmeric crude extract on P-glycoprotein activity in healthy male subjects: a randomized crossover study. J Med Assoc Thai 2018;101:313-21.

be dose-dependent in Caco-2 and L-MDR1 cells[14]. Curcuminoids from a 95% ethanol extract of turmeric rhizome can inhibit P-gp efflux transport in the rat ileum[11]. In addition, a recent study has demonstrated the inhibitory effect of curcumin on P-gp function at the blood-brain barrier, increasing the digoxin level in the rat brain[15].

At present, there is limited knowledge of the interaction between turmeric crude extract and P-gp substrates in humans. Since turmeric crude extract containing curcuminoids is commonly used in medical practice due to its multiple therapeutic benefits and satisfactory safety profiles[16-23], it is prone to be used in patients taking drugs known as P-gp substrates, such as etoposide, doxorubicine, vincristine, cyclosporine, and indinavir[24]. This preliminary study aimed to investigate the effect of turmeric crude extract on P-gp activity in human subjects. It was hypothesized that the inhibition of P-gp-mediated transport by curcuminoids in turmeric crude extract can lead to an increase in plasma concentration of digoxin, a probe of P-gp substrates, in healthy subjects.

Materials and Methods

Study population

Twelve healthy male subjects (aged 20 to 30 years, body mass index 18 to 25 kg/m^2) who had no known underlying diseases were enrolled in the present study. Individuals were excluded if they had 1) a significant abnormal electrocardiographic finding, 2) a resting heart rate of less than 60 beats per minute, 3) a history of cigarette smoking or alcohol drinking, 4) use of medications or food supplement products (including turmeric- and/or curcuminoids-containing products) within two weeks or consumption of turmeric-rich foods within one week of the study, or 5) any significant abnormal laboratory examination findings (i.e., complete blood count, serum electrolyte, serum creatinine, and thyroid function test) at the screening visit.

Sample size determination

Given the expected standard deviation (σ) of 0.7 for PK parameters of digoxin[25] and assumed differenced PK values between two phases (Δ) of 0.6, with the precision and confidence level of 95% (α = 0.05) and 80% power (1-β = 0.8), a sample size of at least 11 subjects would be required. Considering the recommended, minimum number of evaluable subjects according to the Guideline on the Investigation of Bioequivalence[26], the planned sample size of 12 evaluable subjects was chosen to preliminarily assess any possible interaction between turmeric crude extract and digoxin in this study.

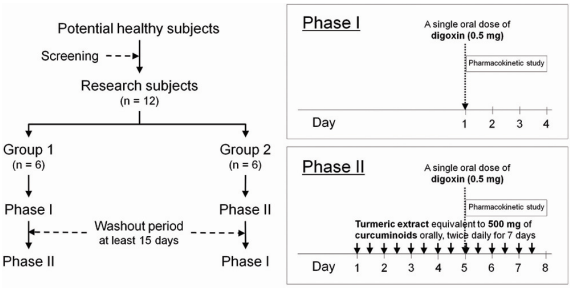

Figure 1. Study design and treatment regimen.

Study design, treatment protocol and safety monitoring

The study design was an open-label, randomized, single-center, two-period, crossover study with a washout period of at least 15 days (Figure 1). Twelve subjects were randomly allocated to one of two groups (six subjects per group). The subjects in Group 1 first participated in Phase I of the study (digoxin alone), then in Phase II (digoxin with concurrent use of turmeric extract), whereas the subjects in Group 2 first participated in Phase II of the study, then in Phase I. The study was conducted at the Clinical Trial Unit of the Faculty of Medicine, Chiang Mai University. The study protocol and related documents were approved by the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University. Each subject gave written informed consent prior to enrollment in the study and undergoing trial procedures.

In Phase I of the study, subjects were admitted to the Clinical Trial Unit. After overnight fasting, they took a single oral dose of two tablets (0.25 mg/tablet) of digoxin (Lanoxin®, GlaxoSmithKline, Boronia, Australia) with 200 mL of water. They then remained in an upright position for at least two hours. Each subject drank 200 mL of water per hour for 12 hours after digoxin administration to achieve a constant urine flow and to minimize renal tubular reabsorption. Vital signs were monitored every hour for 12 hours and electrocardiography [ECG] was performed prior to and at 6 hours and 12 hours following the initiation of digoxin. Three mL blood samples were collected from the forearm by venipuncture through an inserted catheter before starting the study, and then at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, and 12 hours after oral administration of digoxin. Turmeric-free meals were provided to the subjects five hours and 11 hours

after digoxin administration. At 12 hours after taking digoxin, the subjects were discharged from the Clinical Trial Unit. They returned to the study site again at 24, 36, 48, 60, and 72 hours for vital signs monitoring and blood sampling to follow the level of plasma digoxin concentrations after the initiation of digoxin. Adverse events were closely monitored by study investigators and by self-reporting.

In Phase II of the study, each subject took two capsules of turmeric powder extract (GPO-Curcumin®, manufactured by the Government Pharmaceutical Organization of Thailand; each capsule contained turmeric extract equivalent to 250 mg of curcuminoids) in front of study investigators twice daily (1,000 mg/day) after meals for four days prior to admission to the Clinical Trial Unit. On day 5, the subjects were admitted to the Clinical Trial Unit where they took two capsules of turmeric extract with two tablets of digoxin. After the evening meal that day, they took another two capsules of turmeric extract before leaving the Unit. The same dose of turmeric extract was repeated after morning and evening meals (in front of study investigators) for the following two days. Other aspects of the protocol were the same as Phase I of the study.

During the study, subjects had to abstain from caffeine-containing beverages, grapefruit, and grapefruit juice. No other medications were allowed throughout the study period.

Sample preparation and storage

Each blood sample was centrifuged at 1,500 g for five minutes to separate plasma immediately after collection. All plasma specimens were stored frozen at -20°C until analyzed. The determination of plasma digoxin concentrations in all specimens was performed on the same day to minimize inter-day variation of the assay method.

Determination of plasma digoxin concentrations

Plasma concentration of digoxin was determined by the AxSYM Digoxin assay, a microparticle enzyme immunoassay [MEIA]. The Digoxin III assay kits were purchased from Abbott Diagnostics (Abbott Park, IL, USA) and assays were run using an AxSYM analyzer. Samples and all AxSYM Digoxin III reagents required for one test were pipetted by sampling probe into various wells of a reaction vessel [RV]. Sample, anti-digoxin coated microparticles and digoxin-alkaline phosphate conjugate were then combined in the appropriate wells in the RV and immediately transferred to the processing center. Further pipetting was done in the processing center with the processing probe where digoxin present in the sample competed with the digoxin-alkaline phosphatase conjugate for binding with the anti-digoxin coated microparticles to form antibody-antigen and antibody-conjugate complexes. An aliquot of the reaction mixture containing the antibody-conjugate complex was transferred to the matrix cell where the microparticles would bind irreversibly. The matrix cell was then washed to remove unbound materials. Finally, the substrate, 4-methylumbelliferyl phosphate, was added to the matrix cell and the fluorescent product was measured by the MEIA optical assembly.

The Digoxin III assay is linear up to a plasma digoxin concentration of 4.0 ng/mL, while the lower limit of quantification [LLOQ] is 0.30 ng/mL. Any value less than the lower limit of detection of a digoxin assay was considered as "none detected". Any plasma specimen with a value higher than 4.0 ng/mL was manually diluted and the system then calculated the concentration of the sample using the specified dilution factor.

Pharmacokinetic analyses

The PK parameters from each individual plasma concentration-time curve were determined by noncompartmental analysis, using the TopFit software version 2.0 for PC[27]. Maximum plasma concentration [C_{max}] (ng/mL) and time to maximum plasma concentration [T_{max}] (hour) were obtained directly by visual inspection of each subject's plasma concentration-time profile. Area under the concentration-time curve from time 0 to the last quantifiable point [AUC_{0-t}] (ng.hour/mL) was calculated using the trapezoidal rule. AUC from time t to infinity [$AUC_{t-\infty}$] was mathematically integrated by extrapolation of the digoxin concentration from the time of the last quantifiable level to infinity. Total $AUC_{0-\infty}$ is the sum of AUC_{0-t} + $AUC_{t-\infty}$. Since quantifiable digoxin levels in most subjects could be detected only for approximately six to eight hours after digoxin administration (see below), the calculation of the terminal elimination half-life [$t_{1/2\beta}$] (hour) could not be accomplished. Half-life of digoxin [$t_{1/2}$] (hour) was measured from the digoxin plasma concentrations at four hours to six or eight hours.

Data analysis

Data were analyzed using the SPSS version 16.0. The PK parameters are presented as mean ± standard deviation. The differences in the mean values of each PK parameter obtained from both phases were

compared using the paired t-test. A *p*-value less than 0.05 was considered statistical significance.

Comparison between the C_{max} obtained from Phase II (C_{max}.II) and that from Phase I (C_{max}.I) in each subject was performed. Individuals whose C_{max}.II/C_{max}.I ratio was more than 1.25 were classified as Category I (increased C_{max}), individuals whose C_{max}.II/C_{max}.I ratio was between 0.75 to 1.25 were classified as Category II (unchanged C_{max}), and individuals whose C_{max}.II/C_{max}.I ratio was less than 0.75 were classified as Category III (decreased C_{max}).

Results

Twelve healthy male subjects were enrolled in the present study (Table 1). Among them, an oral administration of 0.5 mg digoxin alone (Phase I) achieved the maximum plasma concentration of 2.68±1.22 ng/mL after approximately one hour. Regarding the LLOQ of the digoxin assay, the last quantifiable digoxin level in most subjects was detected at either six hours or eight hours after digoxin administration. The AUC from time 0 to the last quantifiable point (AUC_{0-t}) after the initiation of a single dose of 0.5 mg digoxin was 6.73±1.93 ng.hour/mL, representing approximately 80% of $AUC_{0-\infty}$ (8.51±2.23 hours) (Table 2).

Table 1. Characteristics of study population

	Subjects (n = 12)
Age (year)	22.4±2.4
Height (cm)	172.3±6.3
Weight (kg)	62.1±6.7
Body mass index (kg/m^2)	20.9±1.4
Resting heart rate (beat/minute)	77.3±11.4

Data represent mean ± standard deviation

Table 2. Mean pharmacokinetic [PK] parameters of digoxin obtained from 12 healthy male subjects receiving digoxin alone (D: Phase I of the study) and digoxin with concurrent use of turmeric extract (D + T: Phase II of the study)

PK parameters	D	D + T	*p*-value
C_{max} (ng/mL)	2.68±1.22	3.11±1.25	0.396
AUC_{0-t} (ng.hour/mL)	6.73±1.93	8.41±4.14	0.202
$AUC_{0-\infty}$ (ng.hour/mL)	8.51±2.23	10.79±6.19	0.228
T_{max} (hour)	1.00±0.66	0.85±0.27	0.449
$t_{1/2}$ (hour)	4.89±3.14	6.25±7.10	0.575

C_{max} = maximum concentration; AUC_{0-t} = area under the plasma concentration-time curve from time 0 to the last quantifiable point after digoxin administration; $AUC_{0-\infty}$ = area under the plasma concentration-time curve extrapolated to infinity; T_{max} = time to reach C_{max}; $t_{1/2}$ = half-life (calculated from the digoxin plasma levels at 4 hours to 6 or 8 hours)

Data represent mean ± standard deviation

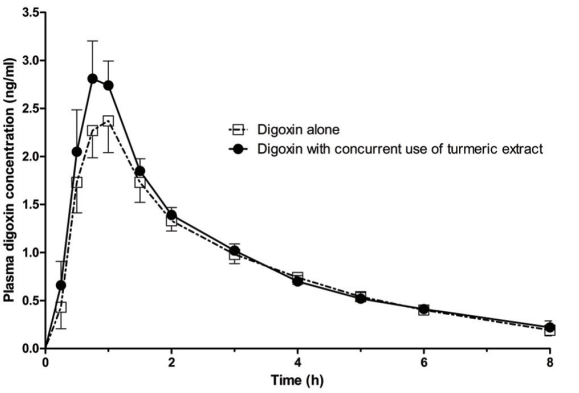

Figure 2. Mean plasma digoxin concentration-time curves from 12 healthy male subjects receiving digoxin alone (Phase I of the study) and digoxin with concurrent use of turmeric extract (Phase II of the study). Error bars represent the standard error of the mean [SEM].

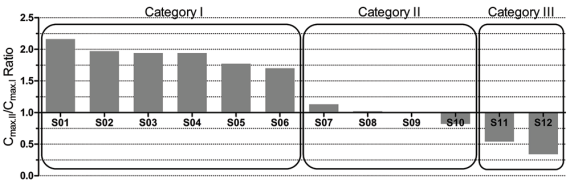

Figure 3. Ratios of maximum plasma concentration of digoxin obtained during Phase II to those from Phase I (C_{max}.II/C_{max}.I) and the number of subjects with ratio values in Categories I, II, and III. Category I = increased C_{max} (C_{max}.II/C_{max}.I >1.25); Category II = unchanged C_{max} (0.75 ≤ C_{max}.II/C_{max}.I ≤1.25); Category III = decreased C_{max} (C_{max}.II/C_{max}.I <0.75).

Compared to Phase I, the values of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ of digoxin in Phase II were somewhat higher, but the difference did not reach statistical significance (Table 1). The mean plasma digoxin concentration-time curves from the 12 subjects receiving digoxin alone and digoxin with concurrent use of turmeric extract are shown in Figure 2.

Close observation of the data among the 12 subjects demonstrated three different categories of turmeric extract and digoxin interaction. With concurrent use of turmeric extract, the C_{max} of digoxin was increased, unchanged, and decreased in six, four, and two subjects, respectively. The ratios of C_{max} in Phase II to those in Phase I for each subject is shown in Figure 3.

Digoxin administration with and without turmeric extract in the present study was generally well-tolerated. Only one subject experienced mild nausea and vomiting at 1.5 hours after the initiation of digoxin during Phase I and recovered without any

medication given. Four subjects had non-pathological ECG changes (T wave inversion and/or flattened T wave) following administration of digoxin either in Phase I (n = 1), in Phase II (n = 1), or in both phases (n = 2). ECG changes in all four subjects returned to normal within 24 hours.

Discussion

Although the concurrent use of turmeric crude extract did not statistically significantly alter the mean PK parameters of digoxin in 12 healthy male subjects, the individual data indicated that it could affect the C_{max} of digoxin in three different directions, increase (n = 6), no change (n = 4), or decrease (n = 2). The 25% change in the C_{max}.II/C_{max}.I ratio of digoxin in each individual subject was considered to be of clinical relevance to differentiate three categories of the interaction because the therapeutic plasma concentration of any P-gp substrate(s) might be altered to toxic or sub-therapeutic concentrations, especially in the case of agents with a narrow therapeutic index[28,29]. These findings suggest that medical professionals should be aware of possible divergent interactions between turmeric crude extract and P-gp substrates for the proper monitoring of their individual patients.

The concurrent use of turmeric crude extract increased the C_{max} of digoxin in half of the subjects in the present study. This finding is in accordance with previous non-clinical evidence[30-32] and one human study using talinolol as a P-gp probe[33]. Since talinolol is the substrate of not only P-gp but other transporters, such as multidrug resistance-associated protein 2 [MRP2] which is abundantly expressed in the human intestine[34], the use of digoxin as a probe to investigate the interaction of drugs through P-gp is preferred[3]. It has been shown that neither blood nor urine levels of curcuminoids can be detected in individuals taking oral curcuminoids at a 500 mg twice daily dosing regimen due to poor absorption of curcuminoids[23,35,36]. Therefore, it is reasonable to postulate that the enhancement of the C_{max} of digoxin in the six subjects was mainly through the inhibition of P-gp activity and/or down-regulation of P-gp expression by curcuminoids in the intestinal tract[37,38]. The assumption that curcuminoids exert their P-gp inhibitory property primarily at the intestinal site is supported by a previous animal study[39]. In rats, oral administration of curcumin (2 or 8 mg/kg) significantly increases the oral bioavailability of etoposide (a P-gp substrate) but does not affect the PK of intravenous etoposide[39].

One-third of the subjects in the present study had an unchanged oral bioavailability of digoxin when turmeric extract was concurrently administered. This might be attributable to genetic polymorphisms of P-gp among the subjects. Low P-gp expression and function of some individuals due to their genetic polymorphisms could potentially contribute to the absence of significant DDI, especially via interference of gastrointestinal absorption between a P-gp substrate and a P-gp inhibitor. He et al demonstrated that oral administration of curcumin (1,000 mg per day for 14 days) in healthy Chinese volunteers led to a significant increase in the bioavailability of talinolol (another P-gp probe) only in the T3435T homozygous group, whereas neither the C3435T heterozygous group nor the C3435C homozygous group had a significant alteration[33]. However, there are discrepancies in the literature related to the significance of the MDR1 polymorphisms for P-gp expression and function. Some papers reported an association between genetic MDR1 polymorphisms and P-gp expression and function, while some did not[40-46]. Based on currently available information, the clinically relevant impact of MDR1 polymorphisms on the PK of P-gp substrates such as digoxin is still unclear[47,48]. Since the analysis of genetic polymorphisms was not performed in the present study, further studies are required. Future PK DDI studies involving P-gp should include genotypic analysis where feasible.

The present study observed a decreased C_{max} of digoxin in two subjects when turmeric extract was concurrently used. This observation was contrary to the study hypothesis which expected a higher C_{max} of digoxin due to the inhibition of P-gp by curcuminoids in turmeric extract. There are, at least, two possible reasons to explain this unexpected contrary finding. First, it might be due to an extensive drug metabolism of curcumin (by CYP1A) in the intestine of the two subjects, leading to low intraluminal curcumin concentration which consequently modulates intestinal P-gp in the opposite way of what was expected[49]. Chearwae et al previously reported that curcuminoids can stimulate the P-gp ATPase activity at low concentrations (0.5 to 1.0 μM) but inhibit P-gp ATPase activity at higher concentrations[12]. Two previous studies conducted by Juan and colleague demonstrated this opposite effect of low-dose curcumin on P-gp activity in healthy Chinese volunteers using talinolol (50 mg) as a P-gp probe[50,51]. Second, the metabolites of curcuminoids (e.g., tetrahydrocurcumin) or other compounds in turmeric extract (e.g., essential oils or

aromatic turmerone) might influence drug disposition by up-regulating the expression and function of intestinal P-gp[51-54]. Hou et al showed that turmeric crude extract could exhibit an effect opposite to curcumin by inducing the expression and function of P-gp in Caco-2 cells[55]. These postulated inductive mechanisms of the unexpected effect of turmeric crude extract on P-gp activity in the two subjects in the present study need further investigations.

Of note, several limitations in this preliminary study could be of value in designing future DDI studies. First, the sensitivity of the method used for detecting digoxin plasma concentration in the present study was limited to a LLOQ of 0.30 ng/mL, leading to insufficient detected values of digoxin concentration during the elimination phase. As a result, the calculation of $t_{1/2\beta}$ could not be accomplished and the effect of turmeric extract on digoxin excretion by the kidneys (if any) could not be determined. A more sensitive and more specific digoxin determination method such as liquid chromatography-tandem mass spectrometry should be considered as a more suitable assay in future studies[56]. Second, analysis of genetic polymorphisms of P-gp was not performed in the present study, resulting in a limited interpretation of divergent results. Future PK DDI studies involving P-gp should take account of genotypic analysis as discussed above. Lastly, the small sample size in this preliminary study possibly correlates with the lack of adequate statistical power to differentiate significant differences of PK values between the two phases.

What is already known on this topic?

Turmeric crude extract is commonly used in medical practice due to its multiple therapeutic benefits. It contains curcuminoids as a major active ingredient. Previous in vitro and in vivo studies demonstrated the P-gp inhibitory property of curcuminoids; however, studies in humans are limited.

What this study adds?

The present study demonstrates divergent effects of turmeric crude extract on P-gp activity in healthy subjects. The concurrent use of turmeric extract with oral digoxin can affect the C_{max} of digoxin, a P-gp probe, in three different ways: increase, no change, or decrease. These preliminary findings suggest that medical professionals should be aware of these possible divergent drug interactions between turmeric crude extract and P-gp substrates for the proper monitoring of their individual patients.

Acknowledgement

The present study was supported by the research grant from the Faculty of Medicine, Chiang Mai University (grant number 052/2557). The authors would like to express their gratitude to Dr. G. Lamar Robert for assistance in editing the manuscript, as well as to Dr. Wanwarang Wongcharoen and Dr. Narawudt Prasertwitayakij for their assistance in reading and interpreting the ECGs of the subjects during the study. Thanks are also extended to Ms. Sujitra Techatoei and Ms. Rapheephorn Khatsri for their secretarial support.

Potential conflicts of interest

The authors declare no conflict of interest.

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