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PHARMACOTHERAPY

The Effect of Food on the Pharmacokinetics of Sildenafil after Single Administration of a Sublingual Testosterone and Oral Sildenafil Combination Tablet in Healthy Female Subjects

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ABSTRACT

Introduction: Female sexual interest/arousal disorder (FSIAD) affects many women worldwide, but pharmacological treatment options are scarce. A new medicine being developed for FSIAD is an on-demand, dual-route, dual-release drug combination product containing 0.5 mg testosterone (T) and 50 mg sildenafil (S), referred to here as T+S.

Aim: The aim of this study was to compare the effect of a fed and a fasted state on the pharmacokinetics of sildenafil following administration of T+S.

Methods: Eighteen healthy women were administered T+S under fed and fasted conditions during 2 separate overnight visits in this randomized, open-label, balanced, 2-period, 2-treatment, 2-sequence crossover study.

Main Outcome Measures: The pharmacokinetics of sildenafil and its active metabolite *N*-desmethyl sildenafil were determined over a 24-hour period. Total testosterone was assessed only at a limited number of time points for quality purposes, as sublingual uptake is not expected to be affected by food intake.

Results: The observed geometric mean ratios (GMRs) and 90% confidence intervals of sildenafil were not all contained within the prespecified bounds (0.80, 1.25). The GMR (90% CI) for plasma AUC_{0-last} was 1.2753 (0.9706−1.6755); for AUC_{0-14h}, it was 1.7521 (1.0819−2.8374); and for C_{max}, it was 1.5591 (0.8634−2.8153). Only lower limits of the CIs fell within the bounds. For *N*-desmethyl sildenafil, the GMR (90% CI) for AUC_{0-last} was 0.8437 (0.6738−1.0564); for AUC_{0-10h}, it was 1.0847 (0.7648−1.5383); and for C_{max}, it was 1.0083 (0.6638−1.5318). Only the GMRs were contained within bounds. No differences were observed between plasma testosterone C_{max} and T_{max} under fed and fasted conditions, which is in line with expectations for a sublingual administration.

Clinical Implications: The T+S combination tablet ruptures too late when taken in a fasted state and should therefore not be taken on an empty stomach.

Strengths & Limitations: This is a well-controlled study that provides important insights into the performance characteristics of the delayed-release coating of the combination tablet. The higher variability of the pharmacokinetic parameters in the fasted state was caused by severely delayed rupture in one-third of the women. A reason for this is proposed but the present data do not explain this phenomenon.

Conclusion: The pharmacokinetics of sildenafil from this modified-release tablet are more robust under fed conditions as compared to the artificial fasted condition where no food is consumed 10 hours prior to and 4 hours after dosing. The dosing situation under the tested fasting condition does not represent the expected common use of this product. Patients should, however, be instructed not to take the tablet on an empty stomach. **Bloemers J, Gerritsen J, van Rooij K, et al. The Effect of Food on the Pharmacokinetics of**

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Key Words: Pharmacokinetics; Testosterone; Sildenafil; Delayed Release; Fixed Dose; Combination Tablet; Female Sexual Interest/Arousal Disorder; Hypoactive Sexual Desire Disorder; Food Effect

INTRODUCTION

Food intake can affect the pharmacokinetics of oral medication. Food effects may change the absorption rate and bioavailability, resulting in drug plasma levels above or below the therapeutic window, changed maximum plasma concentration levels, or a changed time to reach the maximum plasma concentration. The consequences may include serious under- or overdosing or untimely onset or duration of action.[1,2] It is therefore extremely important to investigate the effect of food and, if present, potentially alter the prescribed dose or labeling texts for the drug, depending on the effect. The present study investigates the effect of food on the pharmacokinetic profile of an on-demand medication that has been developed for female sexual interest/arousal disorder (FSIAD).

Low sexual desire in women can cause sexual dissatisfaction and thus negatively affect psychological well-being.[3] If low desire is accompanied by marked distress or interpersonal difficulties, the diagnosis of FSIAD (formerly hypoactive sexual desire disorder) may be applicable, if the complaints are not caused and not better accounted for by the presence of a mental disorder, illicit drug/medication use, or another medical condition.[4,5] Despite FSIAD being a widespread problem,[6,7] the pharmacotherapeutic arsenal is severely lacking, with only 2 drugs approved in the United States, flibanserin (Addyi) and bremelanotide (Vyleesi), and none in Europe. The reason for this lack lies, at least partly, in the fact that drug development strategies have been guided by a "one size fits all" approach that fails to acknowledge the complexity of female sexuality.

Recognizing this complexity, 2 on-demand (ie, pro re nata) drug candidates were designed based on the assumption that FSIAD is caused by at least 2 different etiologies.[8] An on-demand combination tablet containing sublingual testosterone and oral sildenafil (T+S)[9] was developed for a subgroup of women with FSIAD who have a low sensitivity to sexual stimuli. Another on-demand combination tablet containing sublingual testosterone and oral buspirone[10] was developed for women with FSIAD who have dysfunctional overactivation of their sexual inhibitory system. Randomized controlled clinical trials suggest that these drugs may become safe and effective additions to the clinician's arsenal.[11–14]

The window of effect of the T+S combination tablet lasts 3 hours and lies between 3 and 6 hours post dose.[13,14] The timing and duration of this window are determined by the pharmacodynamic (PD) effect of the sublingual testosterone.[15,16] Of note, however, is that the pharmacokinetic (PK) profile of sublingual testosterone precedes and does not overlap its PD profile,[15] with a time to maximum plasma concentration (T_{max}) of 15 minutes and circulating testosterone levels returning to baseline in approximately 1.5 hours.[15,17] But, sublingual testosterone alone is ineffective, which is the reason for the addition of sildenafil in the T+S combination tablet in women with FSIAD/hypoactive sexual desire disorder. The PK and PD effects of oral sildenafil are, in contrast to sublingual testosterone, temporally aligned, as both begin approximately 30 minutes post dose.[18] To ensure the efficacy of the T+S combination tablet, the PD profiles of testosterone and sildenafil must maximally overlap. This means that, following sublingual administration of testosterone, sildenafil must be released into the body after approximately 2.5 hours, and not directly. With this knowledge, a dual-route, dual-release, fixed-dose combination tablet was developed that consists of a testosterone-containing coating for sublingual administration and an inner-core containing sildenafil.[9] A pH-independent, delayed-release coating that is designed to rupture 2.5 hours after dosing surrounds the inner core. This tablet has a PK profile comparable to those of the drugs when administered separately, and it is reported to be a suitable final pharmaceutical drug product.[9]

The present study aimed to investigate the effect of food intake on the pharmacokinetic profile of sildenafil and its active metabolite *N*-desmethyl sildenafil following administration of the T+S dual-route, dual-release, fixed-dose combination tablet. A manuscript describing the effect of food intake on the pharmacokinetic profile of the T+B tablet is in preparation. As T+S is designed for on-demand use, it is important that the release profile is such that the therapeutic window of 3 to 6 hours is not compromised by food intake. Sildenafil absorption is known to be affected by high-fat meals, but this effect is deemed unlikely to be of clinical significance.[18] The delayed-release coating of the inner core is pH independent, and, because sildenafil is released further on in the gastrointestinal (GI) tract due to the delayed-release coating, we do not expect that food effects on sildenafil absorption will be substantial or relevant. The PK profile of testosterone and dihydrotestosterone following sublingual testosterone administration is not expected to be influenced by food intake because sublingual testosterone is directly absorbed systemically via mucosal membranes in the mouth.

METHODS

Study Subjects

Study participants were women between 18 and 55 years of age (inclusive). They were healthy, based on medical history, physical examination, electrocardiogram, and laboratory values,

including vital signs. The women had a body mass index between $\geq$18 kg/m^2 and $\leq$30 kg/m^2, and they had sufficient venous access to allow blood sampling as per protocol. They had to provide written informed consent. Exclusion criteria included endocrine, neurological, and cardiovascular conditions; hypertension; abnormal liver or renal function; and a history of a hormone-dependent malignancy. Medication that could interfere with study medication metabolism or otherwise confound study results was contraindicated, including medications that interfere with sex steroid metabolism, such as oral contraceptives containing antiandrogens or (anti)androgenic progestogens; sildenafil (eg, nitric oxide donor compounds); or testosterone therapy within 6 months before study entry.

Women were recruited via advertisements and a volunteer database. Participants were screened for eligibility approximately 4 weeks prior to study entry, after providing written informed consent. At screening, medical history was recorded, a physical examination including a 12-lead electrocardiogram was performed, a urine pregnancy test was performed, and standard biochemistry, serology, and hematological laboratory parameters were assessed. Baseline levels of total testosterone, sex hormone-binding globulin (SHBG), albumin, thyroid-stimulating hormone (TSH), and follicle-stimulating hormone (FSH) were also assessed at screening. Subject recruitment began on June 23, 2014, and the last visit was on August 13, 2014.

This study was carried out in agreement with the Declaration of Helsinki (264th WMA General Assembly, Fortaleza, Brazil, October 2013) and the International Conference on Harmonization Good Clinical Practice Guidelines for clinical research. It was approved by the Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, the Netherlands; reference number EB96) and by the Dutch Competent Authority (Centrale Commissie Mensgebonden Onderzoek; authorization number NL49313.056.14). It was registered in the European Clinical Trials Database (EudraCT number 2014-001944-38) and was also registered under the Nederlands Trial Register (number NTR4675).

Study Design

This study was a randomized, open-label, balanced, 2-period, 2-treatment, 2-sequence crossover study in healthy female subjects to evaluate the effect of food intake on the pharmacokinetics of sildenafil after a single dose of T+S. In addition, the safety and tolerability of T+S administered after fed and fasted conditions were evaluated. Subjects visited the site twice and stayed overnight (at least 10 hours) during each visit, in an environment controlled for fasting conditions.

For the fed condition, subjects received a high-fat, high-calorie meal (kcal, 900−1000; fat, 60−65 grams; carbohydrates, 60−70 grams; protein, 30 grams) onsite 30 minutes before drug administration. No intake of water was allowed 1 hour prior to and 1 hour following administration of the drug. The drug was taken with 240 mL water to help swallowing, but only after the sublingual administration part. Subjects abstained from food intake the following 4 hours. For the fasting condition, the drug was taken with 240 mL water to help swallowing, but only after the sublingual administration part. No intake of water was allowed 1 hour prior to and 1 hour following administration of the drug. For the next 4 hours the subject abstained from food intake. The food intake was standardized for all subjects for the 12 hours after dosing. Both periods were separated by a washout period of at least 1 week between the dosing of the first period and dosing of the second period.

PK blood samples were taken before dosing and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 14, and 24 hours after drug administration during both visits (totaling 32 PK sampling points). Sildenafil and *N*-desmethyl sildenafil were assessed. Total testosterone levels were measured only for quality purposes at 5 time points because testosterone is directly absorbed systemically via buccal mucosal membranes. After completion of the second period (or after early discharge), the subjects returned to the research unit for a follow-up visit between 7 to 14 days after discharge. Adverse events were queried daily and recorded when spontaneously reported by the subject. Vital signs were checked daily. Lab safety (hematology, biochemistry) and drug and alcohol assessments were performed at site admission and on dosing days.

Medication and Dosing

T+S is a fixed-dose combination of testosterone and sildenafil citrate in tablet form for sublingual followed by oral administration. The outer coating contains testosterone (0.50 mg) that is released immediately after sublingual administration. The inner core of the tablet contains sildenafil (50 mg) and is designed to delay the release of the sildenafil for approximately 2.5 hours; thereafter, the sildenafil is released immediately into the GI tract. Subjects received 2 doses of T+S: one under a fed condition and one under a fasting condition. The order in which a subject received T+S administration under fed or fasting conditions was randomized. Study staff dispensed each tablet, and treatment compliance was ensured by supervised administration. The amount of time that the tablet was held in the mouth was timed so that the tablet was swallowed after 60 seconds. The subjects were instructed to swallow the tablet as a whole, without chewing or otherwise disrupting the dosage form.

Bioanalytical Methods

Sildenafil and *N*-Desmethyl Sildenafil Assay

Plasma samples for sildenafil and *N*-desmethyl sildenafil concentrations and plasma samples for testosterone and dihydrotestosterone (DHT) concentrations were analyzed by Analytical Biochemical Laboratory BV (Assen, the Netherlands). The experiments performed for method validation were based on the U.S. Food and Drug Administration Bioanalytical Method Validation Guidance for Industry. Plasma samples for sildenafil and *N*-desmethyl sildenafil concentrations were assayed using a

validated high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) method with a range of 2.00 to 2,000 ng/mL for both sildenafil and *N*-desmethyl sildenafil in human lithium heparin plasma. Inter-run accuracy and precision for the undiluted (regular) quality control samples were determined at 3 concentrations (6.0, 75.0, and 1,600 ng/mL for sildenafil and *N*-desmethyl sildenafil). Inter-run accuracy and precision were 4.0, 1.7, and 1.7 coefficient of variance (CV%) and −1.1, 1.5, and −0.7 %bias, respectively, for the 3 concentrations of sildenafil, and 4.9, 2.5, and 2.1 CV% and −0.1, 1.9, and −2.6 %bias, respectively, for *N*-desmethyl sildenafil.

The samples were vortex mixed, and 100 μL plasma was transferred into a clean test tube to which 50 μL internal standard solution (400 ng/mL sildenafil-d_8 and 400 ng/mL *N*-desmethyl sildenafil-d_8) was added and vortex mixed. Then, 2.5 mL methyl *tert*-butyl ether was added, and the tubes were capped and rotated for 20 minutes and then centrifuged for 2 minutes at 4000 relative centrifugal force (rcf). The tubes were placed into a cryostatic bath (−45°C), and the bottom water layer was frozen. The supernatant was transferred into a clean tube and evaporated to dryness under a stream of nitrogen at 55°C. The residue was reconstituted with injection solvent and injected for LC-MS/MS analysis. The LC-MS/MS assays were conducted using an Applied Biosystems (Concord, Ontario, Canada) MDS SCIEX API 4000 triple quadrupole mass spectrometer, with positive multiple reaction monitoring and ion spray (turbo spray). The LC system was a Shimadzu (Kyoto, Japan) Prominence SIL-20AC HT autosampler equipped with an Xterra MS C18 3.5-μm column (Waters; Herts, U.K.), with gradients of mobile phase A (200-m*M* NH_4-formiate buffer), mobile phase B (ultrapure water), and mobile phase C (methanol).

Testosterone and DHT Assays

Plasma samples for total testosterone and DHT concentrations were assayed using a validated LC-MS/MS method with a range of 0.0500 to 20.0 ng/mL for both testosterone and DHT in human serum. Inter-run accuracy (%bias) and precision (CV%) for the quality control samples were determined at 3 concentrations (0.118, 1.97, and 15.5 ng/mL for testosterone and 0.129, 2.02, and 15.5 ng/mL for DHT). Results were 10.2, 2.0, and 3.1 CV% and 5.8, −2.3, and −6.3 %bias, respectively, for testosterone and 5.9, 6.6, and 5.1 %CV and −1.6, 3.7, and 7.6 %bias, respectively, for DHT.

The samples were vortex mixed, and 100 μL serum was transferred into a clean test tube to which 50 μL internal standard (4 ng/mL testosterone-d_3 and 8 ng/mL DHT-d_6) was added and vortex mixed. Then, 4.5 mL 20% (v/v) dimethyl ether in *n*-pentane was added, and the tubes were capped, rotated for 20 minutes, and then centrifuged for 2 minutes at 4700 rcf. The tubes were placed into a cryostatic bath (−45°C), and the bottom water layer was frozen. The supernatant was transferred into a clean borosilicate tube and evaporated to dryness under a stream of nitrogen at 40°C. The residue was reconstituted in 500 μL dicholoromethane and derivatized with 2-fluoro-1-methylpyridine. The solvent was evaporated to dryness under a stream of nitrogen at 40°C. The residue was reconstituted in 30% of methanol. The obtained extract was further purified using Waters' Oasis WCX (30 mg, 1 cc) solid-phase extraction cartridges. The final extract was injected on for LC-MS/MS analysis.

Applied Biosystems MDS SCIEX API 4000 triple quadrupole MS, with positive multiple reaction monitoring and ion spray (turbo spray), was used for the HPLC-MS/MS assays. The LC system was a Shimadzu Prominence SIL-20AC HT autosampler equipped with a Kinetex C18, 2.6-μm (3.0 × 100 mm) column, with gradients of mobile phase A (ultrapure water), mobile phase B (100-m*M* NH_4-formiate buffer), and mobile phase C (acetonitrile).

Hormonal Assays Performed at Screening

Standard biochemistry, serology, and hematological laboratory parameters were assessed by KCL Flevoziekenhuis (Almere, the Netherlands). Testosterone at screening was assessed via an Elecsys electrochemiluminescence immunoassay (ECLIA) employing a competition principle with a Modular Analytics E170 module (Roche Diagnostics GmbH; Mannheim, Germany). The lower limit of detection was 0.087 nmol/L. Reference range was <2.9 nmol/L. SHBG was assessed via the Elecsys ECLIA employing a sandwich method using 2 monoclonal antibodies, with the Modular Analytics E170 module. The lower limit of detection was 0.350 nmol/L. The reference range for SHBG was 26.0−110 nmol/L. FSH was assessed via the Elecsys ECLIA employing a sandwich method using 2 monoclonal antibodies, with the Modular Analytics E170 module. The lower limit of detection was <0.100 mIU/mL. The reference range for FSH was 1.5−116 IU/L. TSH was assessed via the Elecsys ECLIA employing a sandwich method using 2 monoclonal antibodies, with the Modular Analytics E170 module. The lower limit of detection was 0.005 μIU/mL. The reference range for TSH was 0.3−4.5 mIU/L. Albumin was assessed via a Roche Cobas colorimetric assay using bromocresol green, with a Cobas c501. The lower limit of detection was 2 g/L. The reference range for albumin was 35−52 g/L.

Statistical Analysis

The present study performed a pharmacokinetic comparison of the effect of food on the pharmacokinetics of sildenafil. The study was not designed to determine bioequivalence. Sample size calculations were not performed. Time to maximum concentration (T_{max}), maximum peak concentration (C_{max}), area under the plasma concentration vs time curve (AUC_{0-inf}, AUC_{0-last}), elimination rate constant (λ_z), and elimination half-life ($t_{1/2}$) for plasma sildenafil and *N*-desmethyl sildenafil were calculated. In addition, the AUC_{0-10h} for *N*-desmethyl sildenafil and

AUC_{0-14h} for sildenafil were determined. Actual times of blood draws were recorded and used for analyses.

For the PK of sildenafil, an analysis of variance of the log-transformed AUC_{0-inf} (AUC_{0-last}) and C_{max} were performed in order to obtain 90% CIs for the ratios after administration of T+S under fed conditions relative to fasted conditions. All other data and parameters were evaluated descriptively only. There were 2 primary endpoints in this study: sildenafil AUC_{0-last} and sildenafil C_{max}. The pharmacokinetic parameters for sildenafil (AUC_{0-last}, AUC_{0-14h}, and C_{max}) following a single dose of T+S in fed and fasted conditions were compared using a linear mixed-effects model appropriate for a 2-period, crossover design. The model contained period and treatment as fixed effects, and subject as a random effect. A log transformation was applied to the AUC and C_{max} prior to analysis. The 90% CIs, based on a *t*-distribution, were generated from the above model for the least-square geometric mean ratios (GMRs, fasted/fed) for the 2 primary endpoints. The 90% CIs were compared to the prespecified standard bioequivalence bounds (0.80, 1.25). If the 90% CIs of all endpoints were contained within the interval (0.80, 1.25), then it would be concluded that the pharmacokinetics of sildenafil in T+S after administration under fasting conditions are likely bioequivalent to administration under fed conditions.

Descriptive statistics for both sildenafil and *N*-desmethyl sildenafil were provided by treatment. Minimum, median, and maximum mean, SD, and CV% were provided for all PK parameters. Plasma testosterone and DHT, as well as vital signs, clinical safety laboratory tests, and adverse events, were evaluated descriptively only. All analyses were performed using Phoenix WinNonlin 6.4 (Certara; Princeton, NJ), and were performed by WIL Research Laboratories ('s-Hertogenbosch, the Netherlands).

Table 1. Baseline characteristics (N = 18)

Parameter	Value
Age (y)	
Mean (SD)	36.6 (10.7)
Median	36.1
Range	19.3–55.6
Age categories (n)	
<40 y	10
40–60 y	8
Weight (kg)	
Mean (SD)	66.3 (6.6)
Median	66.0
Range	53.7–77.0
Menopausal status (n)	
Postmenopausal	4
Premenopausal	14
Contraceptive use (n)	
Hormonal	8
Combined OAC	6
Implanon	1
IUD (Mirena)	1
Non-hormonal (copper IUD, abstinence, or condoms)	6
None (postmenopausal)	4
Race (n)	
Asian	2
African	1
Caucasian	15
Hormones	
Total testosterone (nmol/L)	
>1.7 to <2.2	2
>0.7 to 1.7	6
≤0.7	10
SHBG (nmol/L) (SD)	73.3 (39.0)
Albumin (g/L) (SD)	46 (1.8)

IUD = intrauterine device; OAC = oral contraceptive; SHBG = sex hormone-binding globulin.

RESULTS

A total of 18 women with a mean age of 36.7 years (SD = 10.7) were enrolled in the study between June 24, 2014, and August 13, 2014, at FlevoResearch, Almere, the Netherlands. Demographic characteristics are described in Table 1. One subject was withdrawn before dosing in the crossover period after breakfast (and not replaced) because it proved impossible to place a working cannula. No subjects were withdrawn because of adverse events. Only 2 subjects had 2 measurable concentrations of sildenafil in the fasted condition. For one subject in the fasted condition, the sildenafil sample taken at 60 minutes was excluded from analysis due to possible sample mix-up, labeling error, or contamination. For *N*-desmethyl sildenafil, only 2 subjects had 2 measurable concentrations in the fasted condition, and 3 had no measurable concentrations in the fasted condition. For these subjects, no PK parameters could be calculated, and they were excluded from the respective PK comparison. For some subjects, no accurate half-life for sildenafil or *N*-desmethyl sildenafil could be determined, as the criteria as defined in the analysis plan for reliable half-life calculation were not met (fewer than 3 time points, correlation coefficient lower than 0.9, span of time points used in $t_{1/2}$ was not at least twice the calculated value of $t_{1/2}$). Instead of estimated values of AUC_{0-inf}, exposures expressed as AUC_{0-last}, AUC_{0-14h}, and AUC_{0-10h} were used for comparisons of sildenafil and *N*-desmethyl sildenafil. For 3 subjects in the sildenafil fasted condition, 4 subjects in the sildenafil fed condition, 8 subjects in the *N*-desmethyl sildenafil fasted condition, and 7 subjects

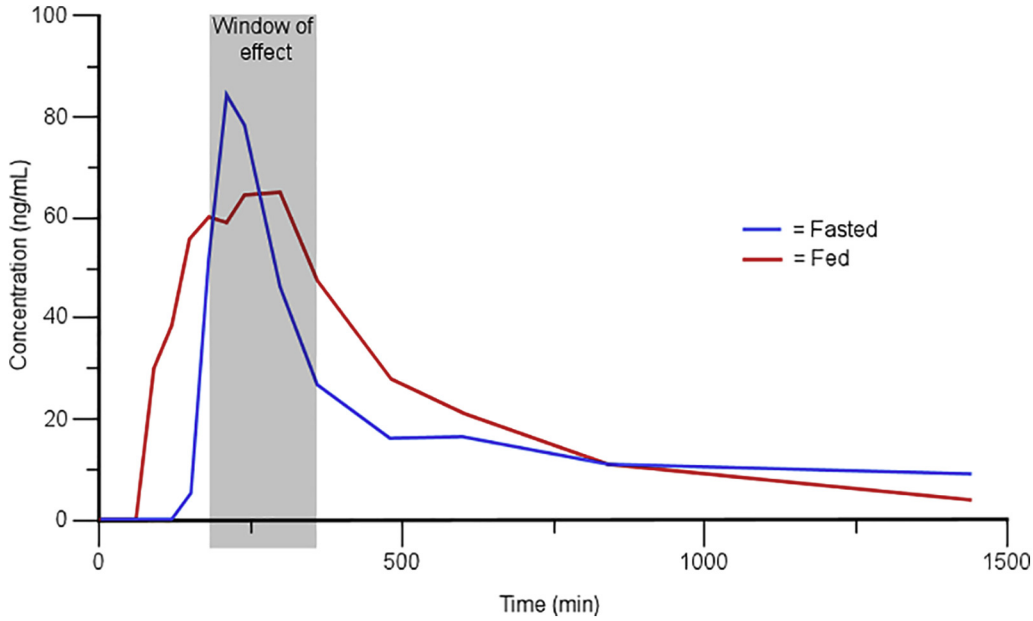

Figure 1. Arithmetic mean plasma concentration/time profile of sildenafil (ng/mL) after administration of T+S (0.5 mg testosterone and 50 mg sildenafil) under fed and fasted conditions in healthy female subjects (linear scale). The gray box indicates the testosterone-dependent pharmacological window of effect, which has been previously described.[13–16]

in the *N*-desmethyl sildenafil fed condition, no reliable half-life could be calculated.

Pharmacokinetic Results

Sildenafil

Figure 1 shows the mean plasma concentration/time profile of sildenafil after administration of T+S. Neither the observed geometric mean ratios (fed/fasted) nor the 90% CIs were contained within the prespecified bounds (0.80, 1.25). The GMRs and associated 90% CIs for the plasma AUC_{0-last}, AUC_{0-14h}, and C_{max} of sildenafil after administration of T+S were 1.2753 (90% CI, 0.9706−1.6755), 1.7521 (90% CI, 1.0819−2.8374), and 1.5591 (90% CI, 0.8634−2.8153), respectively. Specifically, observed GMRs and the upper 90% CIs of the observed GMRs for plasma AUC_{0-last}, AUC_{0-14h}, and C_{max} of sildenafil exceeded 1.25. Thus, AUC_{0-last}, AUC_{0-14h}, and C_{max} of sildenafil were >1.25 times larger in the fed condition than in the fasted condition. However, the lower 90% CIs of the observed GMRs (fed/fasted) for AUC_{0-last}, AUC_{0-14h}, and C_{max} of sildenafil were contained within the bounds (Table 2). For 4 subjects in the fasted state, it took exceptionally long to reach maximum peak exposure of plasma sildenafil (between 14 and 24 hours), which was about 4 to 7 times the median T_{max} of 3.5 hours. Two of these subjects also had very few measurable concentrations of plasma sildenafil. This delayed exposure is reflected in the mean T_{max}, which was 4.1 hours (SD = 1.7) in the fed condition and 8.0 hours (SD = 8.4) in the fasted condition. In addition, the CV% for the AUC and C_{max} in the fasted group (68% and 109%, respectively) was somewhat higher than the variability in the fed group (41% and 69%, respectively) (Table 3). There were no indications that these extreme scores were caused by reasons other than a food effect.

N-Desmethyl Sildenafil

Figure 2 shows the mean plasma concentration/time profile of *N*-desmethyl sildenafil after administration of T+S. The ratios of the least square geometric mean (LSGM) AUC_{last}, AUC_{0-10h}, and C_{max} for the metabolite *N*-desmethyl sildenafil were 0.8437, 1.0847, and 1.0083, respectively. The corresponding 90% CIs were 0.6738−1.0564 for AUC_{last}; 0.7648−1.5383 for AUC_{0-10h}; and 0.6638−1.5318 for C_{max}. So, the LSGMs were within bounds (0.80, 1.25), but none of the 90% CIs were, except for the upper bound of AUC_{last} (Table 4). See Table 5 for a summary of the PK parameters of the primary analysis. The influence of the delayed exposure as observed in the sildenafil analyses is less apparent for *N*-desmethyl sildenafil, as most of the subjects with delayed exposure to sildenafil had no measurable values of *N*-desmethyl sildenafil and thus could not be included in the *N*-desmethyl sildenafil analyses. One subject showed almost no decline in concentrations in the terminal phase, and as a consequence the calculated half-life appeared exceptionally high (34 hours). The variability (CV%) of half-life in the fasted group (104%) was therefore higher as compared with the variability in the fed group (31%) (Table 5).

Testosterone and Dihydrotestosterone

Testosterone and dihydrotestosterone levels were measured at a limited number of time points (5) for quality purposes. No differences were observed between plasma testosterone and DHT C_{max} and T_{max} under fed and fasted conditions, which clearly demonstrates the absence of a possible effect of food on

Table 2. Summary of pharmacokinetic analyses of sildenafil after administration of T+S in healthy female subjects in fed and fasted conditions

	Fed		Fasted		Fed/fasted	
Parameter	N	LSGM	N	LSGM	Ratio	90% CI
AUC_{0-last} (h·ng/mL)	17	474.64	16	372.19	1.2753	0.9706–1.6755
AUC_{0-14h} (h·ng/mL)	17	416.91	16	237.95	1.7521	1.0819–2.8374
C_{max} (ng/mL)	17	100.78	16	64.64	1.5591	0.8634–2.8153

AUC = area under the curve; LSGM = least square geometric means; T+S = 0.5 mg testosterone and 50 mg sildenafil.

sublingual testosterone absorption. The mean C_{max} and T_{max} of testosterone and dihydrotestosterone are presented in Table 6.

Safety

Of the 18 subjects that participated in the study, 15 subjects reported probable drug-related treatment-emergent adverse events (TEAEs). All TEAEs were characterized as mild or moderate in severity. None of the TEAEs led to discontinuation. TEAEs were evenly spread across both fed and fasted conditions. There were no serious adverse events. The adverse events were consistent with those reported for sildenafil. The most common TEAEs were headache and flushing. T+S was well tolerated in both conditions. There were no signs of clinically relevant treatment effects on any of the laboratory parameters. All remained within the reference ranges.

DISCUSSION

The present study showed that, following intake of a single dual-route, dual-release, combination T+S tablet, the mean plasma concentrations as measured by the AUC and C_{max} of sildenafil are higher in the fed condition as compared to the fasted condition. The observed GMRs (fed/fasted) for the plasma AUC_{0-last} and C_{max} of sildenafil after administration of T+S were 1.275 and 1.559 for AUC and C_{max}, respectively, and their 90% CIs were not all contained within the prespecified bounds (0.80, 1.25). Specifically, both the upper limit of the 90% CIs of the observed GMRs for plasma AUC_{0-last} and C_{max} of sildenafil exceeded 1.25, with actual values of 1.675 and 2.815, respectively. Yet, the lower limits of the 90% CIs of the observed GMRs (fed/fasted) for both AUC_{0-last} and C_{max} of sildenafil were well within the bounds. The AUCs for the main metabolite

Table 3. Summary of pharmacokinetic parameters of sildenafil after administration of T+S in healthy female subjects in fed and fasted conditions*

	T_{last}	T_{max}	C_{max}	AUC_{last}	AUC_{0-14h}	AUC_{0-inf}†	λ_z†	$t_{1/2}$†
Treatment	(h)	(h)	(ng/mL)	(h·ng/mL)	(h·ng/mL)	(h·ng/mL)	(1/h)	(h)
Fed								
N	17	17	17	17	17	15	15	15
Mean	19.3	4.1	124.0	508.0	449.0	569.9	0.166	4.67
SD	5.2	1.7	85.4	210.0	187.0	221.0	0.063	1.52
Min	14.0	1.5	43.3	226.0	209.0	259.0	0.085	2.23
Median	24.0	5.0	87.3	444.0	372.0	491.0	0.144	4.82
Max	24.2	8.0	356.0	890.0	811.0	925.0	0.310	8.15
CV%	27.0	42.0	69.0	41.0	42.0	38.8	38.1	32.6
Geometric mean	n/a	n/a	102.0	469.0	414.0	530.1	0.157	4.43
Fasted								
N	16	16	16	16	16	9	9	9
Mean	22.1	8.0	107.0	455.0	354.0	612.4	0.138	5.92
SD	4.0	8.4	116.0	308.0	296.0	369.1	0.068	2.24
Min	14.0	3.0	10.7	104.0	18.7	293.0	0.071	2.35
Median	24.0	3.5	75.3	387.0	289.0	373.0	0.119	5.83
Max	24.2	24.0	468.0	1280.0	1140.0	1360.0	0.295	9.73
CV%	18.0	105.0	109.0	68.0	84.0	60.3	49.3	37.9
Geometric mean	n/a	n/a	64.7	375.0	238.0	528.8	0.126	5.50

AUC = area under the curve; CV% = coefficient of variance; Max = maximum; Min = minimum; n/a = not applicable; T+S = 0.5 mg testosterone and 50 mg sildenafil.

*Only 1 measurable concentration was present for 1 subject (fasted), and only 2 measurable concentrations were present for 1 subject (fasted). All results are rounded to 1 decimal place except for N, half-life, and λ_z.

†Approximation.

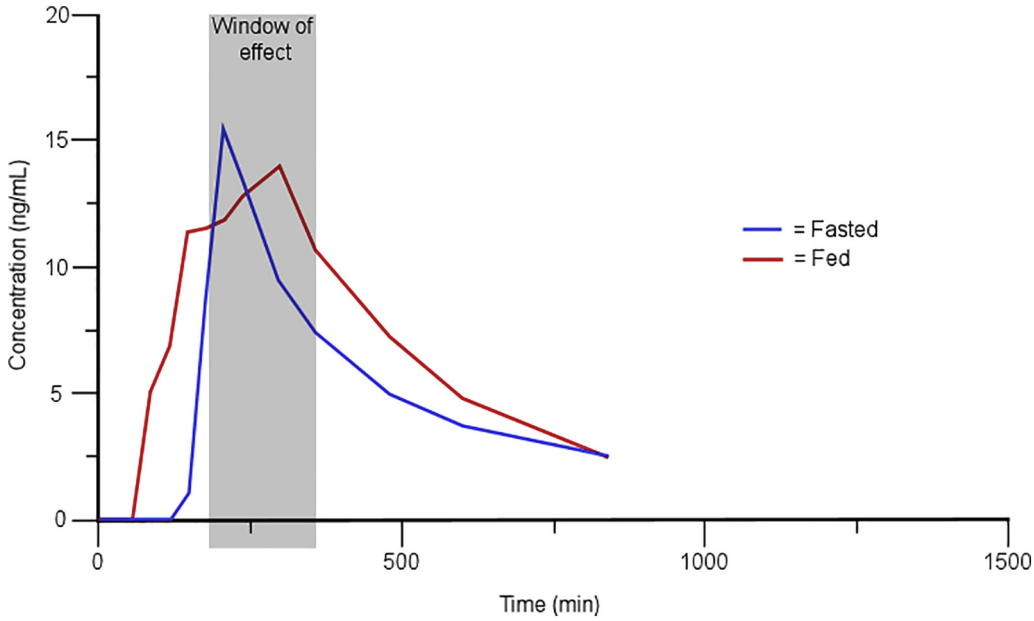

Figure 2. Arithmetic mean plasma concentration/time profile of *N*-desmethyl sildenafil (ng/mL) after administration of T+S (0.5 mg testosterone and 50 mg sildenafil) under fed and fasted conditions in healthy female subjects (linear scale). The gray box indicates the testosterone-dependent pharmacological window of effect, which has been previously described.[13–16]

N-desmethyl sildenafil did not differ between the tested conditions.

It is known that food intake reduces the exposure (AUC) to sildenafil after oral administration. However, this holds for conventional immediate-release formulations of sildenafil, where food intake causes a delay in absorption and subsequently an expected reduced exposure of this high-clearance compound. This effect of food intake on the uptake of conventional sildenafil tablets is not considered to be clinically significant.[18] The currently tested combination tablet does not show a reduction in exposure; instead, an increased exposure is observed in the fed condition. However, this effect was largely determined by 4 women. In these women, it took exceptionally long to reach the maximum peak exposure of plasma sildenafil. This was also true for the 2 women who had only 1 or 2 measurable concentrations and were thus not analyzed; the measurable concentrations were observed in the last and second-to-last drawn blood samples. The delayed exposure in these 6 women was very likely caused by delayed rupture of the coating surrounding the sildenafil-containing tablet core.

With poor absorption, exposure can be higher with food due to higher solubility with the food constituents. When the tablet is properly absorbed, exposure is often higher under fasted conditions than under fed conditions because food can delay and reduce the uptake of these orally administered drugs. In the current experiment with properly absorbable sildenafil citrate, however, AUC and C_{max} were higher in the fed condition. The reason for this inverse effect is likely caused by delayed rupture of the delayed-release coating surrounding the tablet core during the fasted state. This is probably caused by reduced friction on the tablet in the gastrointestinal tract. The delayed-release coating allows water to permeate, thereby softening the core. This creates a malleable tablet with an ever-weakening coating. The rupture of the coating, which is instant, and the ensuing release of sildenafil from the core are partly dependent on the friction the tablet encounters in the gastrointestinal tract. This friction is likely lower in fasted conditions because of decreased contents and decreased peristalsis, as there is less food to be processed. Indeed, in 4 subjects during the fasting condition, it took extremely long for the tablet to rupture and for the first

Table 4. Summary of pharmacokinetic analysis of *N*-desmethyl sildenafil after administration of T+S in healthy female subjects in fed and fasted conditions

	Fed		Fasted		Fed/fasted	
Parameter	N	LSGM	N	LSGM	Ratio	90% CI
AUC_{0-last} (h·ng/mL)	17	90.81	13	107.63	0.8437	0.6738–1.0564
AUC_{0-10h} (h·ng/mL)	17	73.32	13	67.60	1.0847	0.7648–1.5383
C_{max} (ng/mL)	17	19.65	13	19.49	1.0083	0.6638–1.5318

AUC = area under the curve; LSGM = least square geometric mean; T+S = 0.5 mg testosterone and 50 mg sildenafil.

Table 5. Summary of pharmacokinetic parameters of *N*-desmethyl sildenafil after administration of T+S in healthy female subjects in fed and fasted conditions*

Treatment	T_{last} (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{last} (h·ng/mL)	AUC_{0-10h} (h·ng/mL)	AUC_{0-inf}† (h·ng/mL)	λ_z† (1/h)	$t_{1/2}$† (h)
Fed								
N	17	17	17	17	17	12	12	12
Mean	13.4	5.4	23.6	98.6	82.3	121.0	0.214	3.53
SD	3.3	5.1	13.9	36.8	37.5	40.6	0.067	1.07
Min	10.0	1.5	9.2	39.6	36.3	52.1	0.117	2.00
Median	14.0	5.0	18.2	99.7	73.00	133.0	0.202	3.45
Max	24.1	24.1	49.1	162.0	162.0	177.0	0.347	5.95
CV%	25.0	94.0	59	37.0	46.0	33.6	31.3	30.5
Geometric mean	13.1	4.3	20.0	91.3	74.4	113.6	0.205	3.38
Fasted								
N	13	13	13	13	13	11	11	11
Mean	18.0	4.5	23.8	113.0	77.8	158.0	0.137	8.90
SD	6.0	2.9	16.5	51.1	47.0	76.7	0.080	9.21
Min	10.0	3.0	5.1	40.1	12.2	67.5	0.020	2.87
Median	14.0	3.5	21.6	101.0	78.8	170.0	0.174	3.99
Max	24.2	14.0	57.4	205.0	182.0	310.0	0.242	34.30
CV%	33.0	66.0	69.0	45.0	60.0	48.5	58.5	103.5
Geometric mean	17.1	4.0	18.7	101.0	63.8	141.0	0.109	6.37

AUC = area under the curve; CV% = coefficient of variance; Max = maximum; Min = minimum; T+S = 0.5 mg testosterone and 50 mg sildenafil.
*Only 1 measurable concentration was present for subject 1015 (fasted); only 2 measurable concentrations were present for subject 1011 (fasted); and no measurable concentrations were present for subjects 1010, 1014, 1017 (fasted). All results are rounded to 1 decimal place except for N, half-life, and λ_z.
†Approximation.

measurable concentration to be observed, and 2 subjects could not be analyzed because their only measurable concentrations were observed in the last and second-to-last drawn blood samples. Interestingly, the other subjects showed expected rupture times. So, this absence-of-food effect was present in 6 women. This suggests that the effect of food on rupture time is not entirely gradual and likely not dependent on the presence or lack of food alone. Apparently, sometimes the encountered friction is so low that the core remains intact. Future research will have to determine if this delayed rupture during fasting is dependent on the patient (eg, individual differences in peristaltic activity) or on another mediating factor (eg, previous diet).

As with the food intake effect of conventional formulations of sildenafil, the extent to which sildenafil AUC_{0-last} and C_{max} did not fall within bioequivalence requirements is not expected to be clinically significant, but this requires confirmation. The higher observed levels of plasma sildenafil in the fed condition are not expected to result in increased safety risks, as they fall well below circulating levels of sildenafil following the regulatory approved and safe doses of 100 mg. As for efficacy, patients are instructed to have a sexual encounter between 3 and 6 hours after dosing, during the pharmacodynamic window of the combination tablet. During this time, the exposures of sildenafil and its main metabolite are similar in both fed and in fasted conditions, as shown in Figures 1 and 2. A cautionary note, however, is that bioequivalence was not established during this time period. The fasted condition as tested in this study (no food 10 hours prior to and for at least 4 hours after intake) is unlikely to be a very common situation in normal use of this product, nor is the consumption of a high-calorie, high-fat meal just prior to intake. In most cases, patients will have eaten in the preceding hours or will eat in the subsequent hours. The presence of food does seem to make the performance of the tablet more robust. Because 1 in 3 women in the present study showed extremely delayed

Table 6. Mean pharmacokinetic parameters of testosterone and dihydrotestosterone

Parameter	Testosterone				Dihydrotestosterone			
	C_{max} (ng/mL)		T_{max} (min)		C_{max} (ng/mL)		T_{max} (min)	
	Fed	Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted
Mean	4.4	4.6	15.9	15.0	0.52	0.55	28.2	30.0
SD	1.8	1.0	3.6	0	0.18	0.21	5.0	0

exposure due to what is likely to be a fasted-state-induced delayed rupture, additional administration instructions are warranted (eg, tablet should not be taken on an empty stomach).

There was no need to investigate the effect of food intake on testosterone absorption, as testosterone is fully pre-systemically cleared from the gastrointestinal tract and only directly taken up in the systemic circulation via sublingual mucosal transport. The maximum concentration is achieved within approximately 15 minutes, irrespective of dose, and total testosterone, free testosterone, and dihydrotestosterone levels return to baseline levels within approximately 90 minutes.[15,17] This was confirmed in the present study, which clearly demonstrates the absence of a possible effect of food on sublingual testosterone absorption.

There are several limitations of this study. The relatively high variability observed in the fasted condition was not expected, and a larger sample size would have provided more data to better understand this variability. A larger sample size would also have enabled a more dependable calculation of the terminal half-life. Pre- and postmenopausal women were enrolled in this study, but no differentiation was made based on menopausal status in the analyses. It is unlikely that these groups experienced different effects of food on the PK of sildenafil and *N*-desmethyl sildenafil, but it cannot be ruled out.

T+S, administered under both fed and fasted conditions, was well tolerated. No treatment-related serious adverse events were reported, and the reported TEAEs were all mild or moderate in severity and were consistent with the approved labeling for sildenafil. Extremely delayed tablet rupture, as was seen in 6 women during the fasting condition, will impact efficacy. There is no additional safety concern, as exposure remains the same, but the occurrence of adverse events could be delayed proportionally to delayed rupture.

CONCLUSION

The pharmacokinetics of sildenafil in this modified-release tablet are more robust (show less variation) under fed conditions as compared to the artificial fasted condition where no food is consumed 10 hours prior to and 4 hours after dosing of this on-demand drug product. The dosing situation under the fasting condition does not represent the expected common use of this product. In those rare instances, extreme delay in rupture may occur. This can negatively affect efficacy, but there is no safety concern. Additional administration instructions are warranted. Patients should be instructed not to take the tablet on an empty stomach.

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