Bull. Pharm. Sci., Assiut University, Vol. 28, Part 1, June 2005, pp. 137-142.

THE EFFECT OF CIPROFLOXACIN AND CLARITHROMYCIN ON SILDENAFIL ORAL BIOAVAILABILITY IN HUMAN VOLUNTEERS

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السيلدينافيل هو أول وسيلة علاجية عن طريق الفم لعلاج ضعف الانتصاب وكانت الاتاحة الحيوية الفموية لـه 40% فقط وذلك بسبب التخلص منه قبل الوصول الى الدورة الدموية العامة وذلك عن طريق انزيم **CYP_3A_4**. وقد اختبرت هذه الدراسة تأثير تعاطى السبروفلوكساسين أوالكلاريثروميسين التى تثبط عمل انزيم **CYP_3A_4** مع السيلدينافيل. وقد تناول اثنى عشر متطوعا من الرجال الأصحاء السيلدينافيل منفردا أو بعد تناول المثبطات. وقد أظهرت دراسة حركية الدواء أن تعاطى سبروفلوكساسين مع السيلدينافيل قد زاد المساحة تحت المنحنى من (1336±407) الى (2751±968) ميكروجرام ساعة/ لتر كما زادت قيمة أقصى تركيز فى البلازما من (236±71) الى (478±210) ميكروجرام/ لتر. وقد قلت قيمة الاخراج الكلية / الاتاحة الحيوية من (40.1±9.9) الى (20.9±8.6) لتر/ ساعة. كما قلت قيمة الحجم التوزيعى/ الاتاحة الحيوية من (134.9±9.9) الى (71.6±27.4) لتر دون التأثير على معدلات اخراج أو امتصاص الدواء. وقد دللت النتائج على أن تزامن استعمال السبروفلوكساسين أو الكلاريثروميسين مع السيلدينافيل يزيد الاتاحة الحيوية للسيلدينافيل ويعزى هذا التأثير الى المفعول المثبط لكل من سبروفلوكساسين وكلاريثروميسين على انزيم **CYP3A4** ولذلك فانه من الضرورى ضبط الجرعة عند تعاطى السيلدينافيل مع مثل هذه الأدوية.

Sildenafil is the first oral therapeutic agent for the management of male erectile dysfunction. Its oral bioavailability is only 40% due to extensive presystemic elimination, mainly by CYP3A4. This study examined the effect of coadministration of ciprofloxacin or clarithromycin which inhibit CYP3A4 on the bioavailability and pharmacokinetics of sildenafil. Twelve healthy male volunteers received sildenafil alone or after pretreatment with the inhibitors in a balanced three-way crossover design. The pharmacokinetic analysis showed that ciprofloxacin coadministration with sildenafil significantly increased the AUC from 1336±407 to 2751±968 µg hr/L and the C_{max} from 236±71 to 478±210 µg/L. The CL_{tot}/F was decreased from 40.1±9.9 to 20.9±8.6 L/hr and the Vd/F from 134.9±9.9 to 89.1±31.8 L, without affecting sildenafil elimination and absorption rate constants. Similarly, clarithromycin coadministration increased sildenafil AUC from 1336±407 to 2920±666 µg hr/L and C_{max} from 236±71 to 520±176 µg/L. The CL_{tot}/F significantly decreased from 40.1±9.9 to 18.1±5.0 L/hr and the Vd/F from 134.9±9.9 to 71.6±27.4 L, without affecting sildenafil elimination and absorption rate constants. These results indicate that coadministration of ciprofloxacin and clarithromycin significantly increased sildenafil bioavailability which can be inhibitory effect of ciprofloxacin and clarithromycin on CYP3A4. Dose adjustment of sidenafil is thus necessary when administered with such drugs.

INTRODUCTION

Sildenafil is the first oral therapeutic agent introduced for the management of male erectile dysfunction. This drug is a potent selective inhibitor of phosphodiesterase type 5 (PDE5), the predominant isoenzyme responsible for the metabolism of cyclic guanosine mono-phosphate (cGMP). During sexual stimulation the cavernous nerves release nitric oxide which induces cGMP formation and smooth muscle relaxation in the corpus cavernosum. Sildenafil inhibition of the PDE5 mediates a sequence of events starting with elevation in the cGMP, which causes corpus cavernosum smooth muscle relaxation, leading to increase in the blood flow and enhancement in the erectile function.[1,2]

Received in 27/1/2005 & Accepted in 9/4/2005

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The inhibition of PDE5 which present in the systemic circulation can lead to vasodilatation and subsequent reduction in the systolic and diastolic blood pressure.[3] It also produces vasodilatation-related side effects including headache, flushing, rhinitis, dizziness, dyspepsia, and visual abnormalities.[4] Serious cardiovascular events can occur in patients with congestive heart failure and patients with low blood volume and hypotension. Also, patients taking nitrates or other drugs that can increase the systemic exposure of sildenafil may be at risk of developing these serious adverse effects.[2-4]

Sindenafil is rapidly absorbed after oral administration with peak plasma concentration achieved in approximately one hour. The half life of sildenafil is about 4 hours and the plasma protein binding is about 96%. Sildenafil is primarily eliminated from the body by metabolism with only 15% of the bioavailable dose excreted unchanged in urine.[5] Sildenafil undergoes extensive presystemic elimination after oral administration which results in its low bioavailability. This presystemic elimination is mediated primarily by the cytochrome P450 isoenzyme CYP3A4.[6,7] The resulting n-desmethyl metabolite is pharmacologically active and account for about 20% of sildenafil pharmacological activity. Inhibitors of CYP3A4 are expected to increase the plasma concentrations of sildenafil resulting in augmentation of the pharmacological and the adverse effects of sildenafil. It has been reported that coadministration of sildenafil with cimetidine, erythromycin, protease inhibitors and grapefruit juice significantly increased the plasma concentrations of sildenafil in healthy male volunteers. This suggests that lower sildenafil doses are required for patients receiving such drugs.[8-11]

Ciprofloxacin and clarithromycin are known inhibitors of CYP3A4, which have been reported to produce clinically significant interactions with a number of other therapeutic agents that are substrates for this isoenzyme.[12-15] Because of the widespread use of sildenafil and since ciprofloxacin and clarithromycin are widely used antibiotics, it is very likely that some patients may use these drugs simultaneously. The current research was performed to investigate the interaction of sildenafil with ciprofloxacin and clarithromycin. In this study the effect of ciprofloxacin and clarithromycin on sildenafil bioavailability and pharmacokinetics was investigated in normal healthy male volunteers.

MATERIALS AND METHODS

Materials

Sildenafil was obtained from MUP (Ismailia, Egypt) and dexamethasone was obtained from Sigma chemical Co. (St.Louis, MO, USA). Acetonitrile, methanol, and sodium dihydrogen phosphate were purchased from Merck Chemical Co. (Darmstadt, Germany). Diethyl ether was obtained from Honil Limited (London, UK). All chemicals were of analytical reagent grade and all solvents were HPLC grade. The drug products sildenafil (Viagra® 50 mg, Pfizer Egypt, Cairo, Egypt), Ciprofloxacin (Ciprofloxacin® 500 mg, Amriya Pharm. Ind., Alexandria, Egypt), and clarithromycin (Claribiotic® 500mg, Amriya Pharm. Ind., Alexandria, Egypt) were utilized in the study.

Study design

Twelve healthy male volunteers were recruited to participate in this study. The average of the volunteers was 22 years (range 18-26) and the average weight was 75 kg range 64-87 kg). The study protocol was approved by the ethical committee at Tanta University. The nature of the study was explained to the volunteers and a written consent was obtained from each volunteer. All the volunteers had normal kidney and liver functions and were free from any chronic disease such as hypertension, diabetes, hypotension or liver abnormalities. A balanced three-way crossover study with one week washout period between each treatment was employed. Each volunteer received sildenafil tablet (50 mg) alone, ciprofloxacin tablet (500 mg) plus sildenafil tablet 50 mg and clarithromycin tablet (500 mg) plus sildenafil in three different occasions during the three phases of the study.

Pharmacokinetic study

The subjects were instructed not to take any drugs for at least 72 hours prior to and throughout each study period. They were instructed to fast over night for at least 8 hours before drug administration. All the volunteers were given the same meals throughout the study period. On the day of the study, each

volunteer received one tablet of sildenafil (50 mg) either alone, 2-hours after taking one tablet of ciprofloxacin (500 mg) or 2-hours after taking clarithromycin tablet (500 mg). After the washout period, each volunteer received a different treatment until all the three phases of the study were completed. The volunteers were allowed to eat 3 hrs after sildenafil administration. Blood samples were obtained before drug administration (blank), and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours after sildenafil administration. The samples were collected in clean heparinized tubes. Plasma was obtained by centrifugation and was stored at –20° until analysis. Plasma samples were analyzed for sildenafil using an HPLC method.

Sample analysis

Plasma samples were analyzed for sildenafil using the HPLC method developed in our laboratory. A set of clean test tubes were spiked with 50 □l of the internal standard solution,1 □g/ml dexamethazone in methanol, and the methanol was left to evaporate in a water bath adjusted at 50°. To each of these tubes, 0.5 ml of the plasma samples was added and the tube contents were vortex mixed for 1 min. The plasma samples spiked with the internal standard were extracted with 4 ml of ether by mechanical shaking for 3 minutes before centrifugation for10 min. The ether layer in each tube was transferred to a clean test tube and was evaporated in a water bath at 50°. The residue was dissolved in 150 □l of the mobile phase and 50 □l of the resulting solution was injected onto the HPLC.

The mobile phase consisted of 35% acetonitril in 20 mmol sodium dihydrogen phosphate buffer. Separation was achieved at ambient temperature using a reversed-phase column (15 cm x 3.9 mm (i.d.) C_{18}, 4 μm Nova-pack (Waters® Inc., MA, USA) at a flow rate of 1.3 ml/min. The column effluent was monitored by UV detector at 240 nm.

Blank plasma was spiked with the internal standard and known amounts of sildenafil to produce standard samples with concentrations in the range of 50μg/L – 1000 μg/L. Calibration curves were constructed from the obtained peak area ratio and the concentration of sildenafil in each standard sample. The concentrations of sildenafil in the unknown samples were determined from the calibration curves. The assay was fully validated for linearity, selectivity, precision, accuracy and stability. The coefficient of variation for the within-day and between-day accuracy and precision ranged from 1-9%.

Pharmacokinetic analysis

The pharmacokinetic model used to describe the plasma concentration time profile of sildenafil after a single oral administration was one compartment with first-order absorption and first-order elimination. Sildenafil pharmacokinetic parameters were estimated by nonlinear regression analysis using WinNonlin (version 1.5, Scientific Consulting, Inc.USA). The initial parameters estimated by the program were the volume of distribution (Vd/F), total body clearance (CL_{tot}/F), and the absorption rate constant (k_a). The remaining pharmacokinetic parameters such as the elimination rate constant (k), half-life ($t_{1/2}$), time to achieve the maximum plasma concentration (t_{max}), maximum plasma concentration (C_{max}), and area under the curve ($AUC_{0\rightarrow\infty}$) were calculated for the estimated pharmacokinetic parameters.[16]

Statistical analysis

The pharmacokinetic parameters estimated during the three different treatments were compared with each other using the paired t-test. P-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Results

Sildenafil was absorbed rapidly after oral administration reaching a maximum plasma concentration in about 1.5 hr, then the drug was eliminated with a half life of 2.4 hrs. Coadministration of ciprofloxacin with sildenafil resulted in much higher plasma sildenafil concentrations, which was reflected in the significant increase in AUC from 1336±407 μg hr/L to 2751±968 μg hr/L and the C_{max} from 236±71 μg /L to 478±210 μg/L (Figure 1). The pharmacokinetic analysis showed that CL_{tot}/F was significantly reduced from 40.1±9.9 L/hr to 20.9±8.6 L/hr and the Vd/F from 134.9±9.9 L to 89.1±31.8 L. The elimination of sildenafil was also significantly delayed. The elimination rate constant was decreased from 0.337 ± 0.048 hr^{-1} to 0.261 ± 0.042 hr^{-1} and the elimination half life was

prolonged from 2.39 ± 0.231 hr to 3.25 ± 0.369 hr (Table 1).

Coadministration of clarithromycin with sindenafil significantly increased the plasma sildenafil concentration also resulting in significant increase in sildenafil AUC from 1336±407 µg-hr/L to 2920±666 µg-hr/L and C_{max} from 236±71 µg /L to 520±176 µg/L (Figure 2). The CL_{tot}/F was significantly decreased from 40.1±9.9 L/hr to 18.1±5.0 L/hr and the Vd/F from 134.9±9.9 L to 71.6±27.4 L, without affecting sildenafil elimination rate constant and absorption rate constant (Table 1).

Coadministration of ciprofloxacin or clarithromycin with sildenafil produced a non significant effect on the rate of sildenafil absorption. This was apparent from the insignificant change in the absorption rate constant and t_{max}. The results of this study indicated that ciprofloxacin and clarithromycin could increase the extent but not the rate of sildenafil absorption in humans.

Fig. 1: Sildenafil plasma concentration-time profile after a single dose of sildenafil 50 mg alone (▲) and after ciprofloxacin pretreatment (■).
Data are presented as mean ± S.E, n=12

Fig. 2: Sildenafil plasma concentration-time profile after a single dose of sildenafil 50 mg alone (▲) and after clarithromycin pretreatment (■).
Data are presented as mean ± S.E, n=12

Table 1: The Effect of ciprofloxacin and clarithromycin coadminstration on sildenafil pharmacokinetic parameters.

Treatment	Vd/F(L)	k_a (hr^{-1})	K (hr^{-1})	$AUC_{0\rightarrow\infty}$ (µg.hr/L)	$T_{½}$ (hr)	t_{max} (hr)	C_{max} (µg/L)	CL_{tot}/F (L/hr)
Sildenafil	134.9±14.8	1.51±0.357	0.337±0.048	1336±118	2.39±0.213	1.74±0.247	236±20.6	40.1±2.85
Sildenafil+ Ciprofloxacin	89.1*±9.18	2.05±0.174	0.261±0.042	2751*±279	3.25*±0.369	1.26±0.096	478*±60.6	20.9*±2.50
Sildenafil + clarithromycin	71.6*±7.92	1.92±0.339	0.290±0.035	2920*±192	2.82±0.339	1.44±0.187	520*±50.8	18.1*±1.45

Data are presented as mean ± S.E, n=12
*significantly different from control ($p<0.05$).
Vd= volume of distribution, k_a= absorpation rate constant, k= elimination rate constant, $AUC0_{0\rightarrow\infty}$= area under the curve, $t_{½}$= half-life, t_{max}= time required to achieve maximum plasma concentration, C_{max}= maximum plasms concentration, CL_{tot}= total body clearance and F= bioavailability

Discussions

Sildenafil is the most widely used drug in the management of erectile dysfunction. The use of sildenafil is also expected to increase due to its efficacy in the management of other cardiovascular conditions.[17] For this reason, it is very important to ensure the safety of this drug in a variety of conditions, and to study the potential drug-drug interactions with a wide variety of drugs. The current study was performed to investigate the possible interaction between sildenafil and ciprofloxacin or clarithromycin. These drugs were selected because they are widely used antibiotics and are known to inhibit CYP3A4, the major cytochrome P450 isoform responsible for sildenafil metabolism.[12-15]

Sildenafil metabolism in humans is primarily mediated by the cytochrome P450 3A4 isozyme. This low affinity high capacity CYP3A4 is also responsible for sildenafil extensive presystemic metabolism leading to its incomplete bioavailability.[6,7] In the current study we selected two of the commonly prescribed drugs which are known to inhibit CYP3A4. Ciprofloxacin has been shown to inhibit CYP3A4 and affect the pharmacokinetics of drugs that are eliminated by this cytochrome P450 isoform.[12,13] When sildenafil was administered in the volunteers after taking ciprofloxacin there were two fold increase in the AUC and C_{max}. Ciprofloxacin also prolonged the elimination half life of sildenafil by about 35%. Prolongation of the half life can result from decreasing the total body clearance or increasing the volume of distribution of the drug. It is very unlikely that ciprofloxacin which is only 40% bound to plasma protein, can affect the volume of distribution of sildenafil. So, the effect of ciprofloxacin on sildenafil elimination rate constant and half life is probably due to proportional decrease in its total body clearance. Since the small (35%) but significant increase in sildenafil elimination half life cannot account for the two fold increase in the AUC and C_{max}, ciprofloxacin could be considered to affect sildenafil bioavailability to a larger extent. The results clearly indicated that ciprofloxacin significantly increased sildenafil bioavailability and to a lesser extent decreased sildenafil clearance.

Clarithromycin is a commonly used macrolide antibiotic which is known to inhibit CYP3A4. Studies have shown that clarithromycin can affect the bioavailability of drugs such as amprenavir, simvastatin, ropivacaine and repaglinide due to inhibition of CYP3A4.[18-21] In our study sildenafil administration to the volunteers after taking clarithromycin significantly increased sildenafil AUC and Cmax by more than two folds. The elimination rate of sildenafil was not affected by administration of clarithromycin which was indicated from the unchanged elimination rate constant and half life. This indicates that the effect of clarithromycin on sildefafil is mainly due to the increase in sildenafil bioavailability. This increased sildenafil bioavailability is caused by inhibition of its presystemic metabolism.

The results clearly indicate that both ciprofloxacin and clarithromycin significantly increased sildenafil bioavailability, however only ciprofloxacin slowed lower sildenafil clearance. The current study was performed by studying sildenafil pharmacokinetics after a single dose administration of ciprofloxacin and clarithromycin. It is expected that at steady state higher ciprofloxacin and clarithromycin concentrations are achieved and it is possible that these two drugs can affect sildenafil bioavailability and clearance to a greater extent. It is also important to note that all the volunteers participated in our study did not complain of increased adverse effects when they received sildenafil with ciprofloxacin or clarithromycin. The same was true in the interaction study of sildenafil with saquinavir and ritonavir.[10] Although the significant increase in the plasma concentrations of sildenafil was not associated with increased adverse effects in these healthy volunteers, the effect of higher sildenafil plasma concentrations may be different in patients with cardiovascular diseases. So, drug interactions with sildenafil that can increase the plasma sildenafil concentrations can have clinical significance when sildenafil is administered in patients at higher risk of developing adverse effects with the interaction becoming very dangerous with regular administration of the interacting drugs.

In summary concurrent administration of sildenafil with ciprofloxacin or clarithromycin can result in significantly higher sildenafil plasma concentrations. Since the higher sildenafil concentrations can be associated with increased adverse effects, patients who are

taking ciprofloxacin or clarithromycin may need smaller doses of sildenafil. It is recommended that patients at higher risk of developing sildenafil adverse effects and are taking ciprofloxacin or clarithromycin should seek medical advice before taking their sildenafil therapy.

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