Pharmacokinetics (PK) of ethinylestradiol/levonorgestrel with atazanavir/cobicistat.

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ABSTRACT

Background

The combined oral contraceptive pill is the preferred method of contraception for many women. However, women living with HIV often need to dose adjust their contraception due to drug drug interactions with antiretrovirals. The concentration of ethinylestradiol (EE) is increased by unboosted atazanavir (ATV), and decreased by ATV/ritonavir (while progestin exposure is increased and may lead to side effects). Therefore, if an oral contraceptive is administered with ATV/r, it must contain at least 30µg of EE and strict compliance is necessary. However, data on ATV boosted by cobicistat (ATV/c) are not yet available.

Methods

This phase 1, open label, 57 day, cross over, PK study, enrolled healthy female volunteers aged 18-35 years, who were randomized to: i) group 1 received EE/levonorgestrel (LNG) - Microgynon, alone on days 1-21, EE/LNG (21 days) + ATV/c (14 days) in the co-administration phase (days 22-43) and ATV/c alone on days 43-56; ii) group 2 received ATV/c alone on days 1-21, EE/LNG (21 days) + ATV/c (14 days) in the co-administration phase (days 22-43) and EE/LNG alone on days 43-56. Each group underwent intensive PK sampling on days 14, 35 and 56, and EE/LNG concentrations were measured by LC/MS.

Results

Of 16 healthy female volunteers screened, 13 were enrolled (1 was not eligible and 2 withdrew consent for personal reasons) and 6 completed all PK phases (5 withdrew consent because of gastrointestinal adverse events, fatigue or rash). Geometric mean ratios (GMR, with vs without ATV/c) and 90% confidence intervals (CI) of EE C_{max}, AUC, C_{24h} were 1.05 (0.92-1.19), 1.01 (0.83-1.22), 0.75 (0.60-0.93). GMR and CI (90%) of LNG C_{max}, AUC, C_{24h} were 0.83 (0.68-1.02), 0.92 (0.71-1.18), 1.01 (0.73-1.38). No grade 3 or 4 adverse events or laboratory abnormalities were observed in the women who completed the study.

Conclusions

Our findings show that EE C_{24h} decreased by only 25% with ATV/cobicistat (versus 37% with ATV/ritonavir in previous studies). For the first time LNG PK was investigated during

co-administration with cobicistat and no significant changes in its concentrations were measured.

BACKGROUND

Women account for slightly more than half of the world's 36.7 million people living with HIV/AIDS[1] and the majority are of childbearing age. Early and sustained HIV viral load suppression with antiretroviral (ARV) therapy now enables longer, healthier lives in women living with HIV (WLWH), with negligible risks of sexual/perinatal HIV transmission and improved fertility.[2-5] A significant number of WLWH, however, report wanting to delay or avoid pregnancy,[2, 6] meaning that access to safe and reliable contraception in the context of ARV is a critical aspect of HIV care for women and couples alike.[7] The combined oral contraceptive pill (COCP) is the preferred method of contraception for many women worldwide.[8, 9] It is also used to treat pre-menstrual disorders, heavy and painful menstrual bleeding, some benign breast disorders and may be beneficial in some women with acne.[10] However, WLWH are often unable to use hormonal contraception (HC) due to drug drug interactions (DDIs) with ARVs.[11] DDIs may reduce contraceptive efficacy, thereby risking unintended pregnancy;[10, 12] it may also lower ARV efficacy potentially leading to virological rebound, ARV resistance and increased risk of HIV transmission[13] and it may increase drug levels leading to toxicity; all of which have consequences for women's health and for their partners and children.[14, 15] There is now good evidence that there is no risk of sexual HIV transmission when a person living with HIV has achieved sustained viral suppression on ARVs,[5] meaning that many HIV serodifferent couples chose not to use condoms to prevent HIV transmission and rely on a hormonal method for contraception.[2, 16] As WLWH are now in a position to re-evaluate their reproductive choices,[17] it is essential to define the pharmacokinetics (PK) and pharmacodynamics (PD) of individual ARVs co-administered with HC to inform guidelines and ensure that efficacy and safety of contraceptives and ARVs are maintained.

Boosted protease inhibitors (PI/b) such as atazanavir (ATV)/ritonavir have been used for many years and are an instrumental option for third agents in the management of HIV, thanks to once daily dosing and a high genetic barrier.[18] Drug interactions between COCP

and PIs boosted by ritonavir have been described.[17] The concentration of ethinylestradiol (EE) is reported to increase with unboosted ATV (48% increase in area under the curve, AUC),[19] and decrease with ATV/ritonavir (16% decrease in maximum concentration (C_{max},) 19% in AUC and 37% in minimum concentration (C_{min}) of EE).[20] This has been explained by the fact that the increase in exposure of ATV caused by ritonavir (which would be expected to increase the AUC of EE through cytochrome P450 (CYP) 3A4 and CYP2C9 inhibition) is countered by ritonavir's concomitant induction of glucuronidation (via uridine-diphosphate glucuronosyltransferase 1A1 – UGT1A1) responsible for EE clearance.[17]

The exposure of progestins studied to date (norgestimate and norethindrone) increases with unboosted and ritonavir boosted ATV co-administration, which has the potential to lead to side effects.[20, 21]

Therefore, according to guidelines,[22] if an oral contraceptive is administered with ATV/r, it must contain at least 30 µg of EE and strict compliance is necessary.[20] However, data on ATV boosted by cobicistat are not yet available.

Cobicistat is a more recently approved alternative pharmacological booster. Its molecular properties offer the opportunity for co-formulation with PIs into a fixed dose combination[23] and it is available co-formulated with ATV as Evotaz®.[24] It has no ARV activity; however, it is a potent CYP3A4 inhibitor but unlike ritonavir, it is not a UGT1A1 inducer.[23]

The aim of this study was to investigate the steady state PK of EE/levonorgestrel (LNG) 30/150 mcg (Microgynon®) and ATV/cobicistat 300/150 mg (Evotaz®) co-administration in HIV negative female healthy volunteers and to assess its safety and tolerability.

PARTICIPANTS AND METHODS

Participants

Written informed consent was obtained from non-pregnant and non-lactating female healthy volunteers aged between 18 and 65 years with a body mass index (BMI) between 18 and 35 kg/m^2. Participants were excluded if they had any significant acute or chronic medical illness; abnormal physical examination, ECG or clinical laboratory determinations; positive screens for HIV, hepatitis B or C; current or recent (within three months) gastrointestinal disease; clinically relevant alcohol or drug use that the investigator felt would adversely affect compliance with trial procedures; exposure to any investigational drug or placebo within three months of the first dose of the study drug; use of any other drugs, including over the counter medications and herbal preparations, within two weeks of the first dose of the study drug; and previous allergy to any of the constituents of the pharmaceuticals administered during the trial. Women of childbearing potential required a negative pregnancy test at screening and baseline.

Study design

This was an open-label, crossover, 57-day (excluding screening and follow-up) phase 1 PK trial carried out at the Clinical Trial Unit of the St. Stephen's Centre, Chelsea and Westminster Hospital, London, United Kingdom.

At screening, clinical assessment and routine laboratory investigations were performed in all participants. The safety and tolerability of study medications were evaluated throughout the trial (on days 7,14, 28, 35, 49, 56 and at follow-up) using the National Institute of Allergy and Infectious Diseases (NIAID) Division of AIDS table for grading the severity of adult and pediatric adverse events to characterize abnormal findings (2004), vital signs, physical examinations and clinical laboratory investigation.

After successful screening, volunteers were randomized to: i) group 1 received EE/LNG alone on days 1-21, EE/LNG (21 days) + ATV/cobicistat (14 days) in the co-administration

phase (days 22-43) and ATV/cobicistat alone on days 43-56 (14 days); ii) group 2 received ATV/cobicistat alone on days 1-14, EE/LNG (21 days) + ATV/cobicistat (14 days) in the co-administration phase (days 22-43) and EE/LNG alone on days 43-56 (14 or 21 days, patient choice). Each group underwent intensive PK sampling on study days 14, 35 and 56 to measure plasma concentrations of EE/LNG and/or ATV/cobicistat at 0 (pre-dose), 2, 4, 8, 12, 24 hours post dose. On the PK days, study medication intake with a standardized breakfast (626 kcal) and 240 mL of water was witnessed.

Analytical and PK methods

Blood samples were collected into lithium heparin-containing blood tubes (12 mL) at each time-point, immediately inverted several times and then kept on ice or refrigerated until centrifugation. Within 30 minutes of blood collection, each blood sample was centrifuged for 10 minutes at 2000 *g* at 4°C. Plasma was then aliquoted equally into three 2.0 mL tubes (Sarstedt, Germany) and stored at -20°C.

Samples were shipped on dry ice to the Liverpool Bioanalytical Facility for analysis. The laboratory participates in an external quality assurance scheme (KKGT, the Netherlands).

Quantification of ethinylestradiol, levonorgestrel, atazanavir and cobicistat

Concentrations of ethinylestradiol, levonorgestrel, atazanavir and cobicistat in plasma were measured using validated high-pressure liquid chromatography–tandem mass spectrometry methods.[9] The lower limits of quantification (LLQ) for the plasma analyses was 5pg/mL for EE, 0.240 ng/mL for LNG, 10 ng/mL for atazanavir, and 5 ng/mL for cobicistat. For concentrations below the assay limit of quantification, a value of one-half of the quantification limit was used.

Accuracy (percentage bias) was between 0.61% and 3.28% (EE), -0.42% and 1.5% (LNG), 4.70% and 6.36% (ATV), and 6.45% and 8.07% (cobicistat) and precision was less than 8.0% (EE), 3.1% (LNG), 6.3% (ATV), and 8.0% (cobicistat).

Data analysis

The calculated PK parameters for plasma EE, LNG, ATV and cobicistat were the plasma concentration measured 24 hours after the observed dose (C_{24h}), the C_{max} and the AUC from 0 to 24 hours (AUC_{0-24}). All PK parameters were calculated using actual blood sampling time and non-compartmental modeling techniques (WinNonlin Phoenix, version 6.1; Pharsight, Mountain View, CA). Descriptive statistics, including geometric mean (GM) and 95% confidence intervals (95% CI) were calculated for EE, LNG, ATV and cobicistat plasma PK parameters. Each drug PK parameter during the co-administration period was compared to the unaccompanied drug PK parameter by calculating GM ratios and 90% CI (co-administered/alone) using excel.

Inter individual variability in drug PK parameters was expressed as a percentage coefficient of variation [CV, (standard deviation/mean)×100].

ETHICS

The study protocol was approved by the Westminster Research Ethics Committee, London, United Kingdom, as well as by the Medicines and Healthcare products Regulatory Agency (MHRA) in the United Kingdom. The study was conducted according to Good Clinical Practice and the Declaration of Helsinki (EudraCT Number: NCT02697851).

Results

Study population

Sixteen healthy female volunteers were screened, 13 were enrolled (one was not eligible and 2 withdrew consent for personal reasons). Nine subjects completed the intensive PK day 14 (five in group 1, four in group 2), eight completed day 35 (four in each group) and six completed all PK phases (four in group 1, two in group 2). Overall, seven participants withdrew consent; five because of adverse events and two before starting the study medication. In those who completed all PK phases, median (range) age and median BMI were 31 (19-35) years, and 24 (19-29) kg/m^2, respectively. Four participants described themselves as Caucasians, six as black African, and one as Hispanic. Participant demographics, withdrawals and withdrawal reasons are summarized in Table 1.

Pharmacokinetic results

Pharmacokinetic data for all four drugs are summarized in Table 2.

- *Ethinylestradiol plasma pharmacokinetics*

EE GM plasma concentration versus time curves, with and without ATV/cobicistat, are shown in Figure 1. Geometric mean ratios (GMR, with versus without ATV/cobicistat) and 90% CI of EE C_{max}, AUC_{0-24}, C_{24h} were 1.05 (0.92-1.19), 1.01 (0.88-1.22), 0.75 (0.60-0.93).

- *Levonogestrel plasma pharmacokinetics*

LNG GM plasma concentrations versus time, with and without ATV/cobicistat are shown in Figure 2. GMR (90% CI) of LNG C_{max}, AUC_{0-24}, C_{24h} were 0.83 (0.68-1.02), 0.92 (0.71-1.18), 1.01 (0.73-1.38).

- *Atazanavir plasma pharmacokinetics*

GMR (90%) CI of ATV C_{max}, AUC_{0-24}, C_{24h} were 0.75 (0.60-0.95), 0.78 (0.64-0.96), 0.89 (0.72-1.11).

- *Cobicistat plasma pharmacokinetics*

GMR (90% CI) of cobicistat C_{max}, AUC_{0-24}, C_{24h} were 0.88 (0.8-0.97), 0.85 (0.77-0.95), 0.89 (0.66-1.21).

Safety and tolerability

Five participants withdrew consent from the study secondary to side effects; of those, data on the reason are available for three, and are listed in Table 1. No grade 3 or 4 adverse events or laboratory abnormalities were observed in the women who completed the study.

DISCUSSION

To our knowledge, this is the first study to investigate the PK of a combined contraceptive pill co-administered with ATV/cobicistat. Microgynon (EE/LNG), used in our study, is the leading contraceptive pill prescribed in the UK.[25]

The estrogens and progestogens (some of which are ingested as inactive prodrugs) in oral contraceptives undergo extensive first-pass metabolism by phase I and II microsomal enzymes in the small intestinal mucosa and liver before reaching the systemic circulation,[26, 27] leading to a significant susceptibility to DDI. EE is predominantly metabolised by CYP3A4 and CYP2C9 but glucuronidation pathways also play a role in clearance.[28] Our findings show that EE C_{24h} decreased by only 25% with ATV/cobicistat compared with 37% with ATV/ritonavir in previous studies.[20] This may be explained by the fact that, unlike ritonavir, cobicistat does not induce UGT1A1 (nor CYP2C9).[23] There was no reduction seen in AUC nor C_{max}. The EE component of the COCP, as well as inhibiting follicular development of ovulation, is responsible for endometrial stability and a significant reduction in levels can lead to breakthrough bleeding,[11] itself likely to impact on adherence to contraception and risks of pregnancy.

Our study is the first to assess the PK of LNG during co-administration with cobicistat and we found no significant changes in its concentration (GMR C_{24h} 1.01). LNG is a second generation progestin[17] whose main metabolism is through hydroxylation and glucoronidation, which cobicistat is not thought to affect. This is important because an important factor in the efficacy of the combined OCP is the progestogen-mediated suppression of the luteinizing hormone (LH) surge (although the minimum level of progestin needed for efficacy is not completely known).[29, 30] Decreases in

progestin levels could therefore potentially impact contraceptive efficacy. Conversely, increases in concentrations (as seen with ritonavir boosted ATV) may lead to toxicity such as nausea, weight gain and acne, which in turn can impact adherence. LNG is the progestin contained in one brand of emergency contraceptive pill; the fact that no changes in drug concentrations were seen with ATV/cobicistat is therefore reassuring, and consistent with the knowledge that LNG is not subject to any first-pass effect.[31] It is a substrate of glucuronosyltransferases[32] and co-administration with cobicistat would not affect this metabolic pathway, while ritonavir might induce it. Finally, it is worth mentioning that although the latter metabolic pathway is well known, ATV did not lead to an increase of LNG concentrations.

In both groups, ATV concentrations remained above the *in vivo* suggested minimum effective concentration for for wild type HIV (MEC = 150 ng/mL).[18]

There are limitations to this study. The subjects were HIV negative healthy volunteers. As such, PK or pharmacodynamic conclusions cannot be robustly drawn and the practical implications of these PK observations are unknown. Clinical outcome data are required in large cohorts of HIV infected participants, and studies investigating pharmacodynamics endpoints (such as failure of viral suppression, HIV-related clinical disease progression or unintended pregnancy) are needed in order to draw definite conclusions on how likely a contraceptive is to fail in the context of a particular ARV combination. It is also important to remember that efficacy rates of user dependent contraception differ between perfect use (as seen in a clinical trial) and real life use.

Possibly also related to the fact that this study involved healthy volunteers, the drop out rate was high. Five/11 participants withdrew consent due to side effects, which

included a rash and gastrointestinal symptoms. In a real-life clinical setting, the onset of COCP or ARVs is commonly associated with mild side effects, which do not normally persist beyond three to six months, at which point an alternative is usually offered. This option to persist and assess was not available in the context of this study. As a result, the numbers of participants with a complete data set was low. Nevertheless, this is the first study to offer PK data on EE/LNG and ATV/cobicistat co-administration. We demonstrated a lesser decrease in EE with ATV/cobicistat than seen with ATV/ritonavir and no changes in LNG concentrations. This data is important in informing physicians who need to discuss and chose safe and reliable contraception with their female patients living with HIV.

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Transparency declarations

EE has received speaking and travel grants from Janssen, ViiV, Bristol-Myers Squibb, Merck Sharp & Dohme, and Gilead.

EB no conflicts of interest to declare.

LE no conflicts of interest to declare.

SDP

SK has received support from ViiV Healthcare, Merck, Janssen, Gilead and Bristol Myers Squibb for the HIV drug interactions website, and research grants from Merck, Janssen and ViiV Healthcare.

GM has received speaker's and advisor's fees from Gilead Sciences, MSD, Janssen, BMS and has served as a member of the board of directors and on the scientific advisory board of Tobira Therapeutics.

NN

MB had received travel and research grants from and has been advisor for Janssen, Roche, ViiV, Bristol-Myers Squibb, Merck Sharp & Dohme, Gilead, Mylan, Cipla, Teva.

Legends to Tables and Figures

Table 1: Participant demographics, withdrawals and withdrawal reasons.
ID = study identification, BMI = body mass index, G = group, D = day, n/a = not available.

Table 2: Summary of pharmacokinetic data for all four drugs.
EE = ethinylestradiol, LNG = levonorgestrel, ATV = atazanavir, CV = coefficient of variation, GM = geometric mean, GMR = geometric mean ratio, CI = confidence interval, C_{max} = maximum concentration, AUC_{0-24} = area under the curve from 0 to 24 hours, C_{24h} = concentration at 24 hours post-dose.

Figure 1: Ethinylestradiol (EE) geometric mean (GM) plasma concentration versus time curves with and without atazanavir (ATV)/cobicistat.

Figure 2: Levonorgestrel (LNG) geometric mean (GM) plasma concentration versus time curves with and without atazanavir (ATV)/cobicistat.

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