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Metabolic Disposition of [^{14}C] Ulipristal Acetate in Healthy Premenopausal Women

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Abstract

Introduction: Ulipristal acetate (UPA) is a novel selective progesterone receptor modulator for the treatment of benign gynaecological conditions such as uterine myoma. The disposition of [^{14}C] UPA was determined in five healthy premenopausal women after administration of a single oral dose of 20 mg (59 µCi).

Materials and Methods: The single dose study allocated 5 healthy women of reproductive age to receive a single radio labelled dose of 20 mg (59 µCi) UPA. After dosing, blood, plasma, urine and faecal samples were collected for up to 11 days and analysed for concentrations of radioactivity. UPA metabolite profiles in plasma were determined by high-performance liquid chromatography with radioactivity flow detection; metabolite structures were confirmed by liquid chromatography-mass spectrometry.

Results: UPA was rapidly absorbed, exhibiting a mean peak plasma concentration of 141 ng/mL at 0.7 hours post-dose. Plasma radioactivity maxima were observed 0.9 hours post-dose at 281ng equivalents/mL. The total mean recovery of the radioactive dose in excreta was 78.8%, with the majority recovered in faeces (72.5%) and only a small fraction (6.4%) in urine. UPA was extensively metabolised. Radio-chromatograms of plasma revealed that oxidative demethylation was the major metabolic pathway, most likely via cytochrome P450 isoenzyme3A4. At peak plasma radioactivity, the majority of circulating radioactivity was constituted by parent (58.0%), N-monodemethylated UPA (PGL4002 (20.5%)) and N-didemethylated UPA (PGL4004) eluting together with PGL4002+2H (8.3%). Unchanged UPA was not present in faeces, but PGL4002, hydroxylated PGL4004 and UPA +2H, UPA +2O -2H, as well as acetylated or glucuronidated UPA were identified.

Conclusion: After oral administration in healthy premenopausal women, UPA was rapidly absorbed, extensively metabolized via oxidative demethylation, and excreted predominantly in faeces.

Keywords: Metabolite identification; Metabolite profiling; Pharmacokinetics; Mass balance; Ulipristal acetate

Introduction

Ulipristal acetate (UPA) belongs to the class of Selective Progesterone Receptor Modulators (SPRMs) [1]. It has a steroidal structure, which is depicted in Figure 1. Its primary pharmaco dynamic property is to reversibly block the progesterone receptor in its target tissues (uterus, cervix, ovaries, hypothalamus). SPRMs express agonist or antagonist activities to progesterone based on the target tissue and the absence or presence of progesterone [2-4]. Due to these pharmacodynamic properties, UPA is a novel treatment of benign gynecological conditions such as uterine myoma. Two phase III studies evaluating the efficacy of UPA in the treatment of uterine myoma at a maximal dose of 10mg/day were ongoing when this radiolabelled absorption, metabolism and excretion (AME) study was initiated [5,6].

Previous studies of UPA metabolism found that in isolated human microsomes assays, no significant metabolism of UPA was observed in incubations conducted in the absence of nicotinamide adenine dinucleotide phosphate (NADPH). This suggested NADPH-dependent cytochrome P450 (CYP) involvement in the metabolism pathway. UPA was metabolised to two compounds [1], identified as mono-demethylated (PGL4002) and di-demethylated UPA (PGL4004) (Figure 1; PregLem, data on file). The same study also investigated the *in vitro* blood/plasma partitioning of UPA, showing 18.7% of UPA was distributed to the blood cells; plasma protein binding was 98.2% [1].

Absorption, relative bioavailability and CYP3A4 interactions of UPA have been assessed in an open, non-randomized, two period study conducted in healthy premenopausal women under fasting conditions. Subjects received single oral doses of UPA (20 mg) either alone or concomitantly with a moderate CYP3A4 inhibitor (erythromycin propionate) at steady state. Administration of UPA with the CYP3A4 inhibitor led to a limited increase in maximum concentration (C_{max}) (1.2-fold) and a 3-fold increase in systemic exposure (AUC) for UPA. There was also a decrease in C_{max}, and an increase in exposure coupled to a prolonged elimination for PGL4002. This indicated that inhibition of CYP3A4 impacted the rate and extent of absorption of UPA and also its metabolism by slowing the elimination of PGL4002 (PregLem, data on file).

Absorption and dose proportionality have also been evaluated in premenopausal women who received multiple oral doses of 10, 20, or 50 mg of UPA in a randomized, double-blinded, parallel group trial for 10 days. The C_{max} was dose-dependent and was reached within 1 hour (h). The half-life of UPA was between 38 and 49 h. UPA

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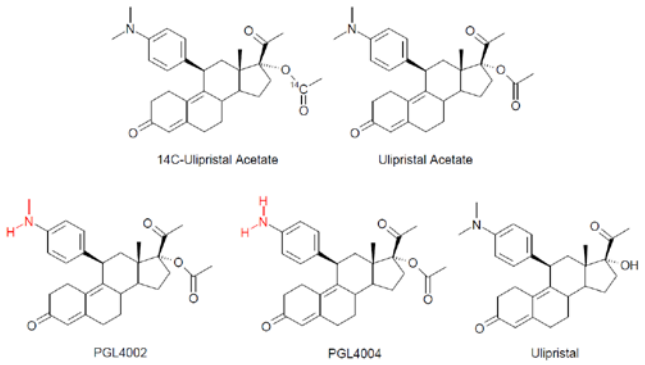

Figure 1: Structure of [^{14}C] Ulipristal acetate, Ulipristal acetate and its oxidative metabolites PGL4002 and PGL4004, and Ulipristal.

exhibited supra-proportional pharmacokinetics, with no unexpected accumulation. Plasma concentrations (C_{max}) were slightly increased at steady state (approximately 1.4-fold), which was reached by day 8 [7].

In this AME study, we assessed the pharmacokinetics and metabolic disposition of UPA in healthy premenopausal women after oral administration of 20 mg (59 μCi) of [^{14}C] UPA. The radioactive doses that are administered to volunteers in Phase I radiolabelled AME studies are tightly regulated by local and governmental agencies [8-10]. Guidance by the World Health Organization and International Commission on Radiological Protection usually restrict radiation exposure to between 0.5 to 1 mSv of radioactivity per subject [11,12], typically equating to a radioactive dose of up to 3.7 MBq (100 uCi). The permissible radioactive dose for this study was set to 59 uCi, since the predicted residence of the radioactivity in the body was expected to be long based on the slow excretion of radioactivity during a quantitative whole body autoradiography study in rats (PregLem study PGL09-019, data on file). Administration of a relatively low amount of radioactive compound, blended with a therapeutic dose of non-radio labelled compound defines the specific activity of the administered material which in turn will govern analytical limits of detection and success in metabolite profiling and identification.

Materials and Methods

Compounds and reagents

[^{14}C] UPA, labelled in the 17α-acetyl moiety (Figure 1) was prepared in a one-step synthesis from UPA precursors by Almac Sciences (Craigavon, UK). PGL4002 was provided by PregLem SA (Geneva, Switzerland) and ulipristal (Figure 1) was obtained from HRA-Pharma (Paris, France). All solvents and reagents were analytical or high-performance liquid chromatography (HPLC) grade. Solvents and general reagents used for sample extractions and HPLC mobile phase preparation, including methanol, acetonitrile, and acetone were obtained from VWR International Ltd (Lutterworth, UK), Rathburn Chemicals Limited (Walkerburn, UK), Sigma-Aldrich Company Ltd (Gillingham, UK) and Vickers Laboratories Ltd. (Pudsey, UK). Permafluor E+ and Ultima-Flo Mscintillation fluids were obtained from PerkinElmer LAS Ltd (Beaconsfield, UK).

Study design

This open-label phase I study designed to evaluate the metabolism and disposition of [^{14}C] UPA after a single oral dose was conducted at a single investigational study site (Covance Clinical Research Unit Ltd., Leeds, UK). Five healthy ambulatory premenopausal women were enrolled in the study. On day 1 of the in-patient phase (day –1 to day 10), subjects were administered a 20 mL ethanol:water solution (50% v/v) containing 20 mg [^{14}C] UPA with an activity of 59.0 μCi and a radiochemical purity of at least 97%. This equated to an estimated radiation exposure of 0.5 mSv. The dose was administered with water (200mL), and subjects abstained from food or drink (except water) for at least 10 h before and 4 h after dose administration. At the time of [^{14}C] UPA administration, complete vital signs, clinical laboratory evaluations and a 12-lead ECG were obtained, and blood, urine, and faecal samples were collected. Physical, laboratory, and ECG evaluations were also performed on day 10. Subjects were discharged from the centre when a mass balance cumulative recovery >90% had been achieved or <1% was recovered in urine on two consecutive days. At the anticipated date of discharge (day 10), it was observed that the thresholds described above were not met, and therefore subjects were asked to return to the centre for additional non-residential visits for blood, urine and faecal sampling on days 11 and 12 and on days 17 and 24 (± 1 day) for supplemental urine and faecal collections, when required.

The protocol was approved by the Medicines and Healthcare products Regulatory Agency and the Independent Ethics Committee before the start of the study, which was conducted according to the "Declaration of Helsinki" and ICH GCP consolidated guidelines. Permission to perform the study was also granted by the Administration of Radioactive Substances Advisory Committee.

Sample collection

Venous blood samples were collected at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48 and 72 h after administration of [^{14}C] UPA and then once daily until day 12 (total volume, approximately 480 mL). Blood samples were collected in 12-ml tubes containing lithium heparin, which were centrifuged in a refrigerated centrifuge within 1 h of collection. The resulting separated plasma for each sample was transferred to 5 labelled polypropylene tubes, one for analysis of plasma total radioactivity, two aliquots were analysed for UPA and PGL4002, and the remaining plasma was retained for metabolite profiling and identification.

Whole blood was collected in 2-ml tubes containing lithium heparin as the anticoagulant and analysed for whole blood total radioactivity. Samples were either stored refrigerated (2-8°C) (whole blood radioactivity) or stored frozen at –20°C (plasma radioactivity) or –70°C (UPA/PGL4002 levels, metabolite profiling and identification) pending analysis.

Urine samples were collected before [^{14}C] UPA administration, at specified intervals after dose administration (-24h–0, 0–4, 4–8, 8–12, 12–24 and 24–48 h), and once daily over 24 h until day 12 and once on days 17 and 24, when necessary.

All urine voided during each specified interval was pooled and refrigerated (2-8°C) after the total volume and collection time was recorded. Faecal samples and bathroom tissues for each bowel movement were collected individually over each 24-h interval after administration of [^{14}C] UPA and stored at –70°C (with the exception of additional non-residential collections) until analysis.

Measurement of radioactivity

Plasma, whole blood, urine, and faeces were analysed for total radioactivity using liquid scintillation counting to determine the

pharmacokinetics and mass balance by Covance Laboratories (Harrogate, UK). Concentrations of radioactivity were determined in plasma using duplicate 500-µL aliquots, which were directly assayed for radioactivity after addition of 10 ml of Permafluor E+ scintillation fluid (PerkinElmer LAS Ltd, Beaconsfield, UK) using a liquid scintillation counter (Packard Tricarb models 1900, 2050 and 2100 liquid scintillation counters with facilities for computing quench corrected counts per minute (cpm)). Background radioactivity was subtracted before computation of results. The limit of detection was taken as 2 times the background radioactivity determined by counting samples with no radioactivity in the same batch as the samples. A Packard oxidizer was used for combustion of blood and faecal homogenate samples. Duplicate aliquots of whole blood (500 µL) were submitted for combustion analysis. Faecal samples were weighed, pooled by subject for each 24-hour collection period and homogenized in an appropriate volume of deionised water. Duplicate aliquots (500 mg) of each homogenate were combusted.

Measurement of unchanged UPA and PGL4002 in plasma

Concentrations of unchanged UPA and PGL4002 in plasma were determined by SGS Cephac (Saint-Benoît, France; data on file) using a validated liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) assay.

Over the calibration range 0.100 to 20.0 ng/mL, the overall precision and accuracy of the assay were 4.0-9.8% and -0.8-1.0% for UPA, respectively. Over the same calibration range, overall precision and accuracy of the PGL4002 assay were 1.5-4.6% and -3.0-2.8%, respectively.

Metabolite profiles in plasma, faeces and urine

Due to the low concentrations of radioactivity in the systemic circulation, metabolite profiles in plasma were analysed by off-line detection. HPLC eluate was collected in 12 second fractions using a fraction collector, dispensed into 96-well Lumaplates, and subsequently analysed using a Top Count Microplate system (with NXT v2.14 software; Lablogic Systems Ltd, Sheffield, UK). Data from TopCount was re-imported into the Laura software package (version 3.4.7.52 SP8; Lablogic Systems Ltd, Sheffield, UK) to generate profiles for evaluation.

Initially, 20 samples of plasma (4 from each of the five subjects) at time points up to 24 hours were prepared for analysis. A suitably sized aliquot of each individual sample (between 0.4 g and 1.0 g) was vortex mixed with 3 volumes of acetonitrile and stored at approximately 2-8°C for approximately 1 hour to allow the precipitation of proteins. The samples were centrifuged at 3000 rpm at 4°C for 10 minutes. The resulting supernatant (Extract 1) was transferred to a clean vessel and the pellet was extracted with a further 3 volumes of acetonitrile. Following centrifugation, the supernatant was combined with Extract 1. The combined supernatant was evaporated to near-dryness. The residue was reconstituted in a suitable volume of HPLC mobile phase A (0.1% formic acid in HPLC water) and the recovery of radioactivity was then determined.

Following the individual analysis of the plasma samples, an assessment of the chromatograms resulted in the decision to pool samples in order to improve the likelihood of metabolite identification. To improve sensitivity, the 1 h (C_{max}) plasma samples from all 5 subjects were pooled by equal volume to prepare a single plasma sample for extraction and analysis. Following extraction and concentration, the extract was resuspended in approximately 500 µL mobile phase prior to HPLC analysis.

Individual samples of urine at 4 h were analysed following centrifugation to remove particulate matter. Due to the low levels of radioactivity present, a single pooled urine sample was prepared for each individual, comprising equal volumes (0.1% by weight) of samples over the range of 8 to 264 h.

Metabolite profiles in faeces were generated using HPLC with on-line radioactivity detection and individual faecal extracts were initially analysed. The faeces samples chosen for the identification work were those that covered the range of radio- metabolites including the sample containing the greatest recovery of radioactivity in a single sample, and a sample pool that would contain the remainder of the radio-metabolites present from that subject. Due to the low levels of radioactivity present, a single pooled faeces sample was prepared for each individual, comprising between 0.5% and 2.0% of each wet homogenate weight. A suitably sized aliquot of each pooled faeces sample (between 1.2 and 3.2 g) was extracted with 5 volumes of acetonitrile and placed on a shaker surrounded by water ice for approximately 1 h, to aid the extraction. The samples were then centrifuged at 3000 rpm at 4°C for 10 minutes. Following centrifugation, the resulting supernatant (Extract 1) was transferred to a glass vessel.

High pressure liquid chromatography

Extracted and concentrated residues were typically reconstituted in up to 1000 µL of mobile phase starting conditions; of this, a 25 µL aliquot was removed for recovery calculations, and up to 400 µL was injected onto the high-performance liquid chromatography system. Unlabelled standards of UPA and metabolites PGL4002 and ulipristal (demethylated UPA and de-acetylated UPA, respectively) were added before injection. While fractions were collected, UV profiles (262 nm) for unlabelled standards were monitored to match the retention times of the peaks in the radio-chromatograms. Fractions were collected every 12 sec, and samples were assayed by Top Count. HPLC was performed with a ThermoFisherAccela HPLC or Agilent 1100 HPLC system, which consisted of HPLC column housing maintained at 25°C and a fixed wavelength UV detector. For on-line measurements, a beta-RAM radioactivity flow detector was used to detect radioactivity. The scintillation fluid (Ultima-Flo M, PerkinElmer LAS Ltd, Beaconsfield, UK) was pumped at 2 mL/min for all on-line radioactive HPLC assays using a linear gradient with a mobile phase system consisting of 0.1% formic acid in water and 0.1% formic acid in acetonitrile and was pumped at 1 mL/min through a Inertsil ODS-3 column (4.6×250 mm, 5 µm; Capital HPLC Limited, Broxburn, UK). Laura software was used for data acquisition and processing. The lower limit of detection for radio-chromatography was taken as 3 times the background (approximately 50 cpm for on-line detection, and 10 cpm for off-line detection). The identities of the metabolites were confirmed by liquid chromatography (LC)/mass spectrometry (MS).

Mass spectrometry

A ThermoFisher LTQ Orbitrap hybrid mass spectrometer (Thermo Fisher Scientific, Loughborough, UK) equipped with an electrospray ionization interface and operated in the positive ionization mode was used for metabolite characterization. Settings for the mass spectrometer were as follows: scan range, 100-850 *m/z*; source voltage, 4.5 kV; capillary temperature, 310°C. Mass spectral data for UPA and its metabolites were recorded in full scan mass spectrum, selected ion monitoring, and selected reaction monitoring modes.

Metabolite identification

Two samples of pooled faeces, one individual urine sample and

one pooled 1h plasma were injected on the Orbitrap LC/MS. Due to the low level of radioactivity, the search for metabolites was performed by searching for potential metabolite ions using Metworks software (Thermo Scientific, San Jose, USA). Metworks is an application manager software tool used to interrogate the data in order to look for metabolites of known biotransformations. The MS data in this study were interrogated for possible metabolites including UPA, PGL4002, PGL4004 and ulipristal as well as transformations such as e.g.demethylation, dehydrogenation, reduction, methylation, hydroxylation and glucuronidation.

Calculations

The total radioactivity (cpm) excreted in the urine or faeces were determined by multiplying the volume of urine or the weight of faecal homogenates by the radioactivity concentration of each sample (in cpm/mL or cpm/g, respectively). The dose recovered was determined by total disintegrations per minute in the sample at any given time point, divided by total radioactivity (in cpm) of the dose received by each subject and multiplied by 100. Total concentrations of radioactivity in blood and plasma were determined using a specific activity of the [^{14}C] UPA in the dose formulation administered to the subjects.

Pharmacokinetic analysis

Plasma concentrations of UPA for each subject were analysed using empirical, model-independent methods. Thus, values for C_{max} and time to C_{max} (t_{max}) were taken directly from the observed data. The area under the plasma concentration–time curve to the last quantifiable sample (AUC_{0-t}) was determined using the linear trapezoidal method. The AUC from time zero to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-\infty} = AUC_{0-t} + (C_t/k_e)$, where C_t was the observed concentration of drug for the last quantifiable sample. The terminal rate constant (k_e) was estimated by log-linear regression analysis on data points visually assessed to be on the terminal log-linear phase. The terminal half-life ($t_{1/2}$) was calculated as $t_{1/2}=\ln 2/k_e$. All pharmacokinetic calculations were performed using WinNonlin (version 4.0.1; Pharsight, Mountain View, USA).

Statistical analysis

All subjects received the same treatment and thus no statistical comparisons were performed. The mean ± standard deviation (SD) for concentrations of radioactivity, concentrations of UPA and its metabolite, and UPA pharmacokinetic parameters are presented.

Results

Subjects

All five subjects enrolled in the study were healthy premenopausal women. Each subject received a single dose of [^{14}C] UPA (59 µCi). All women completed the study and were included in the analyses. The mean ± SD age of the subjects was 48 ± 4 years (range, 44-53 years), and the mean ± SD weight and height were 70.2 ± 5.9 kg (range, 61.9-76.6 kg) and 163 ± 10 cm (range, 150-174 cm), respectively.

Absorption and elimination of radioactivity, UPA and PGL4002

The plasma time course profile of radioactivity concentrations indicated rapid absorption and slow elimination of [^{14}C] UPA after oral administration (Figure 2). By 72 h after administration, concentrations of radioactivity in plasma (21 ng Eq/mL) were less than 10% of the concentrations seen at 1 h (229 ngEq/mL). The total radioactivity concentrations in whole blood paralleled those of plasma, but at lower levels, indicating limited distribution of drug-related products into blood cells. The blood/plasma ratio of radioactivity was generally below 0.60 with a slight increase in the ratio after 36 h, suggesting some association of UPA related material with red blood cells at the later time points. The concentrations of UPA and PGL4002 in plasma were determined using a validated analytical method. Table 1 summarizes selected plasma pharmacokinetic parameters of UPA, PGL4002 and total radioactivity in subjects. UPA was rapidly absorbed in this population of premenopausal women; a mean peak concentration of 141ng/mL was achieved at 0.7 h after dosing. Concentrations of UPA declined in an apparent multiphasic manner; elimination of UPA was slow, with a mean ± SD half-life of 52 ± 9 h. Similarly, PGL4002 had a mean peak concentration of 39 ng/mL at 1.0 h after dosing, a multiphasic decline of plasma concentrations and a mean ± SD half-life of 37 ± 15 h. The systemic exposure to PGL4002 ($AUC_{0-\infty}$) was approximately 33% of the UPA exposure. Based on the mean AUCs of unchanged UPA and total radioactivity, parent drug accounted for approximately 6% of the total radioactivity in plasma, suggesting that UPA was almost completely metabolised after oral administration in women.

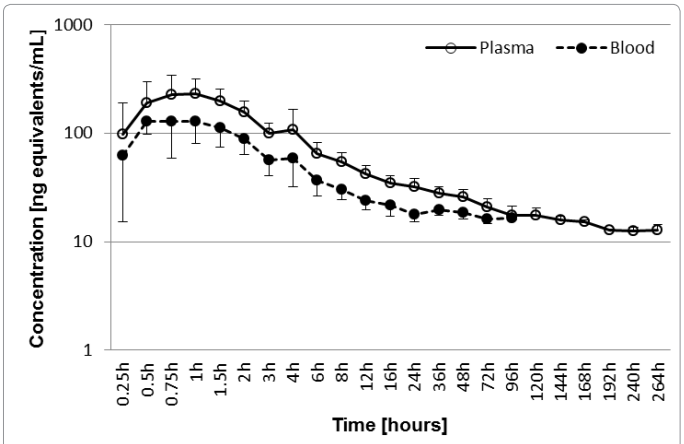

Figure 2: Mean concentrations and standard deviation of total radioactivity in plasma and whole blood following a single oral administration of [^{14}C] Ulipristal acetate to healthy female human subjects at a nominal dose of 20 mg (n=5) Mean concentrations and standard deviation were only calculated when at least 3 values were available.

Pharmacokinetic Parameter	Ulipristal Acetate	PGL4002	Plasma Total Radioactivity	Whole Blood Total Radioactivity
C_{max} (ng/mL or ngeq/mL)	141 ± 31.0	39 ± 8.1	281 ± 41.7	157 ± 34.3
t_{max} (h)	0.7 ± 0.48	1.0 ± 0.33	0.9 ± 0.38	1.0 ± 0.33
$t_{1/2}$ (h)	52 ± 9.1	37 ± 15.1	133 ± 65.4	279 ± 120
AUC_{0-t} (h·ng/mL or ng eq/mL)	423 ± 107	136 ± 44	4733 ±1508	2299 ± 796
$AUC_{0-\infty}$ (h·ng/mL or ngeq/mL)	438 ± 117	143 ± 48	7265 ± 2618	9074 ± 3205

C_{max}: peak concentration; t_{max}: time to C_{max}; $t_{1/2}$: terminal half-life; AUC_{0-t}: area under the plasma concentration–time curve to the last quantifiable sample; $AUC_{0-\infty}$: area under the plasma concentration–time curve extrapolated to infinity.

Table 1: Pharmacokinetic parameters of ulipristal acetate, PGL4002 and total radioactivity in healthy premenopausal women (n=5) after a single 20-mg dose. Data are presented as mean ± standard deviation

Elimination of radioactivity

Eleven days after administration of a single dose of [^{14}C] UPA (59 µCi), the mean total recovery of radioactivity from the combined excreta (urine and faeces) was 79 ± 4%. The fraction of the radioactive dose excreted in each time interval is summarized in Table 2. The rate of excretion was variable, with 0 to 58% of the radioactive dose recovered in faeces within 72 h. The major route of excretion of radioactivity was the faeces, with 73 ± 5% recovered after 11 days. Excretion in urine represented a minor route of elimination of radioactivity, with 6 ± 2% recovered after 11 days. The time course of the mean cumulative excretion of radioactivity is shown in (Figure 3).

Metabolite profile

Individual sample radio-chromatograms did not show a clear radio-profile. A radio-chromatogram of pooled plasma at 1 h after administration of [^{14}C] UPA is shown in Figure 4. The recovery of radioactivity in the pooled 1 h (UPA C_{max}) plasma extract was more than 80% and allowed metabolite profiles to be generated. At all other time points, concentrations approached the lower limit of detection for radio-chromatography and could not be evaluated for metabolic profiling. Not surprisingly, the chromatograms revealed that unchanged UPA and PGL4002 were major components accounting for 58% and 21%, respectively. PGL4004 was a minor component eluting together with PGL4002 + 2H (8%). Small amounts of PGL4004 downstream metabolites (PGL4004 + O and PGL4004 + 2H) and hydroxylated UPA were present at concentrations of 6%, 4% and 3%, respectively. No other metabolites were apparent by radio-chromatography.

Urine and faeces samples did not lend themselves to successful metabolite profiling work, due to the low levels of radioactivity present, and the poor extractability of the radioactivity from the endogenous material.

Mass spectrometric analysis of UPA metabolites

The mass spectral characteristics of an authentic standard of UPA were examined for comparison with metabolites. In the LC-MS spectrum of UPA, a protonated molecular ion ([M + H]+) was observed at m/z 476.28. Structurally diagnostic product ions were observed at m/z 416.26, 277.16, 398.25, 374.25, 358.22, 306.19, 295.17 and 288.17. Changes in the m/z values for these ions provided information for structural elucidation. The major route of biotransformation of UPA was the oxidative demethylation of the 11β-dimethyl aminophenyl group to PGL4002 and PGL4004. Table 3 provides a summary of the

Time [hours]	Dose excretedin 0-264 hours [%]		
	Urine	Faeces	Total
0-4 h	1.15 ± 0.134	-	-
4-8 h	1.14 ± 0.320	-	-
8-12 h	0.65 ± 0.205	-	-
12-24 h	0.98 ± 0.328	-	-
0-24 h	3.92 ± 0.787	1.93 ± 1.691	5.07 ± 1.690
24-48 h	1.02 ± 0.317	2.91 ± 5.014	2.78 ± 3.927
48-72 h	0.50 ± 0.136	28.7 ± 22.82	23.47 ± 23.498
72-96 h	0.30 ± 0.094	16.0 ± 20.53	9.94 ± 16.907
96-120 h	0.23 ± 0.083	11.2 ± 10.01	9.13 ± 10.053
120-144 h	0.15 ± 0.071	7.76 ± 4.745	7.93 ± 4.790
144-168 h	0.13 ± 0.054	2.38 ± 1.318	2.00 ± 1.636
168-192 h	0.10 ± 0.030	4.33 ± 6.919	3.50 ± 6.279
192-216 h	0.09 ± 0.026	9.77 ± 17.90	9.84 ± 17.920
216-240 h	NC	4.69 ± 9.510	4.68 ± 9.510
240-264 h	NC	0.60 ± 0.539	0.60 ± 0.539
Subtotal	6.35 ± 1.616	72.50 ± 4.574	78.80 ± 3.606

Table 2: Mass balance of radioactivity after a single oral dose of [^{14}C] ulipristal acetate in healthy premenopausal women (n=5).
Data are presented as mean ± standard deviation
NC: Not Calculated

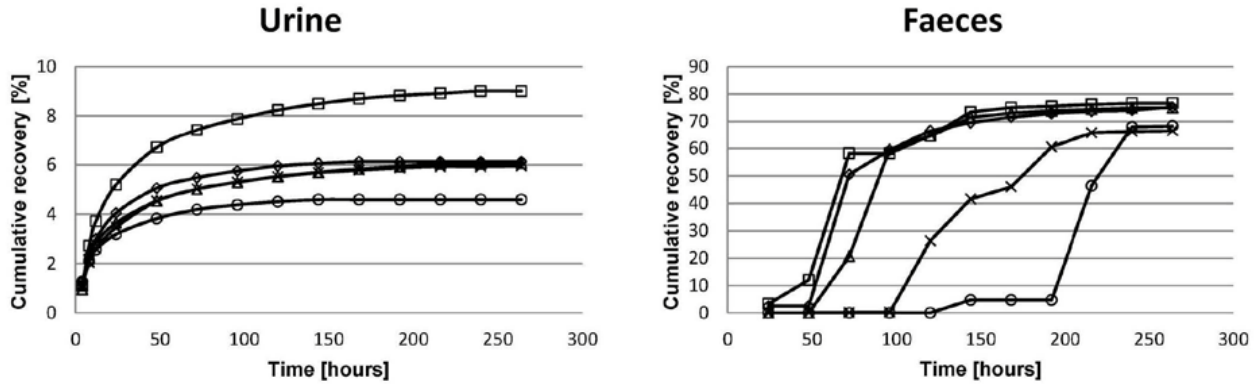

Figure 3: Cumulative recovery of radioactivity per subject following the single oral administration of [^{14}C] ulipristal acetate at a nominal dose of 20 mg (n=5).

metabolite peaks identified in plasma and faeces by LC-MS analysis.

Safety

Four subjects reported a total of 15 adverse events. The majority of adverse events were mild in severity and resolved without treatment. No serious or severe adverse events were reported and no discontinuations from the study occurred. Gastrointestinal disorders such as constipation, abdominal pain or flatulence (5 AEs), fatigue (3 AEs) and pain in extremity (3AEs) were the most reported events. All other AEs only had one isolated incidence and comprised symptoms such as headache, nocturia, vaginal discharge and pallor. There were no clinically significant changes in clinical laboratory evaluations, vital signs, physical findings or any other observations related to safety.

Discussion

UPA, a new SPRM in development for the treatment of uterine myoma, was well tolerated by all five women given a single dose of 20 mg, and no discontinuations or serious adverse effects were observed. After test article administration, there were no effects on clinical laboratory values, vital signs, or ECGs. None of these subjects had any illnesses at baseline that might have interfered with the pharmacokinetics of the test article or the interpretation of the results. The 17α-acetyl labelled UPA was metabolically stable, as previously seen in pre-clinical animal studies (PGL-H-425 and PGL-H-426; PregLem data on file); the formation of ulipristal was not observed. UPA displayed straightforward pharmacokinetic properties after a single oral dose of

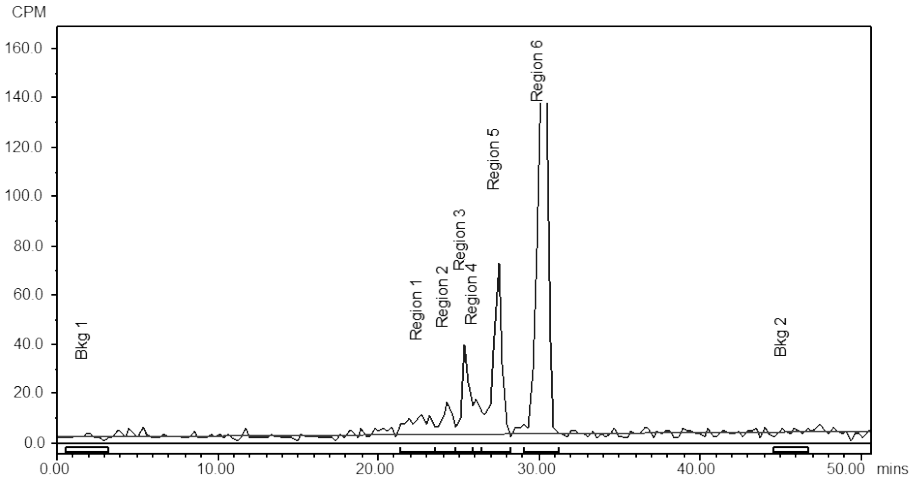

Figure 4: Radio-chromatogram of pooled plasma extract at 1 hour after administration of [^{14}C]-PGL4001 at a nominal dose of 20 mg (n=5)
Regions 1, 2 and 4 corresponding to the minor plasma metabolites and regions 3, 5 and 6 corresponding to major plasma metabolites.
CPM: Counts per minute; mins: HPLC retention time in minutes; Bkg 1/ Bkg 2: Background radioactivity levels;

Metabolite	Rt [min]	*m/z*	Proposed Formula
Major metabolites in plasma			
PGL4004 (didemethylated UPA)	26.39	448.25	$C_{28}H_{34}O_4N_1$
PGL4002 (monodemethylated UPA)	29.77	462.26	$C_{29}H_{36}O_4N_1$
UPA	33.41	476.28	$C_{30}H_{38}O_4N_1$
Minor metabolites in plasma			
Hydroxylated PGL4004	23.01	464.24	$C_{28}H_{34}O_5N_1$
PGL4004 +2H	24.31	450.26	$C_{28}H_{36}O_4N_1$
PGL4002 +2H	26.39	464.28	$C_{29}H_{38}O_4N_1$
Hydroxylated UPA	27.43	492.28	$C_{30}H_{38}O_5N_1$
Metabolites in faeces			
Hydroxylated UPA +Glutathioneconjugate	21.96	781.35	$C_{40}H_{53}O_{10}N_4S_1$
UPA +2H	22.68	478.29	$C_{30}H_{40}O_4N_1$
AcetylatedUPA	25.83	518.29	$C_{32}H_{40}O_5N_1$
PGL4002	30.83	462.26	$C_{29}H_{36}O_4N_1$
UPA +2O -2H	31.01	506.25	$C_{30}H_{36}O_6N_1$
Hydroxylated PGL4004	--*	464.24	$C28H34O5N_1$

* indicates that no radio-trace was available;
Rt: retention time (radio-detection); *m/z*: mass-to-charge ratio
Table 3: Liquid chromatography/mass spectrometry identification of ulipristal acetate (UPA) metabolites.

[^{14}C] UPA in this study. Following administration of [^{14}C] UPA, UPA was rapidly absorbed, with peak plasma concentrations of unchanged UPA, PGL4002 and radioactivity reached at approximately 1 h post-dose. The mean plasma elimination half-life of unchanged drug and PGL4002 was long (approximately 52 h and 37 h, respectively). The pharmacokinetics of UPA and PGL4002, including the UPA to PGL4002 ratio, observed in this study was similar to those seen in previous studies at the same dose [7,13]. Levels of radioactivity declined slowly in plasma and blood with drug-derived radioactivity presenting a low level of blood cell binding.

Analysis of 1 hour human plasma samples by radio-HPLC demonstrated that 6 major regions of interest were present. Three major peaks were identified as UPA (58%), PGL4002 (21%) and PGL4004/PGL4002+2H (8%, eluting together). The three minor components were hydroxy PGL4004 (6%), PGL4004+2H (4%) and UPA+2H (3%). The major route of UPA biotransformation was via sequential demethylations of the 11β-dimethylaminophenyl group to PGL4002 and PGL4004 most likely via the CYP3A4 [1].

Other metabolites that were only present at lower levels in plasma were formed by a series of hydroxylations. Hydroxylated UPA was present at only low levels in plasma.

In the faeces, full scan accurate mass interrogation allowed the identification of the presence of an O-glutathione conjugate of UPA, acetylation of UPA, and UPA +2O -2H at low levels and UPA +2H; however, there was no available radio trace. Further identified faeces components were PGL4002 and hydroxylated PGL4004. The proposed pathway for metabolism of UPA is shown in Figure 5.

The metabolic disposition of UPA in women reported here is consistent with findings reported previously in other SPRMs possessing the same 11β side chain such as mifepristone, lilopristone, onapristone or telapristone acetate [14,15].

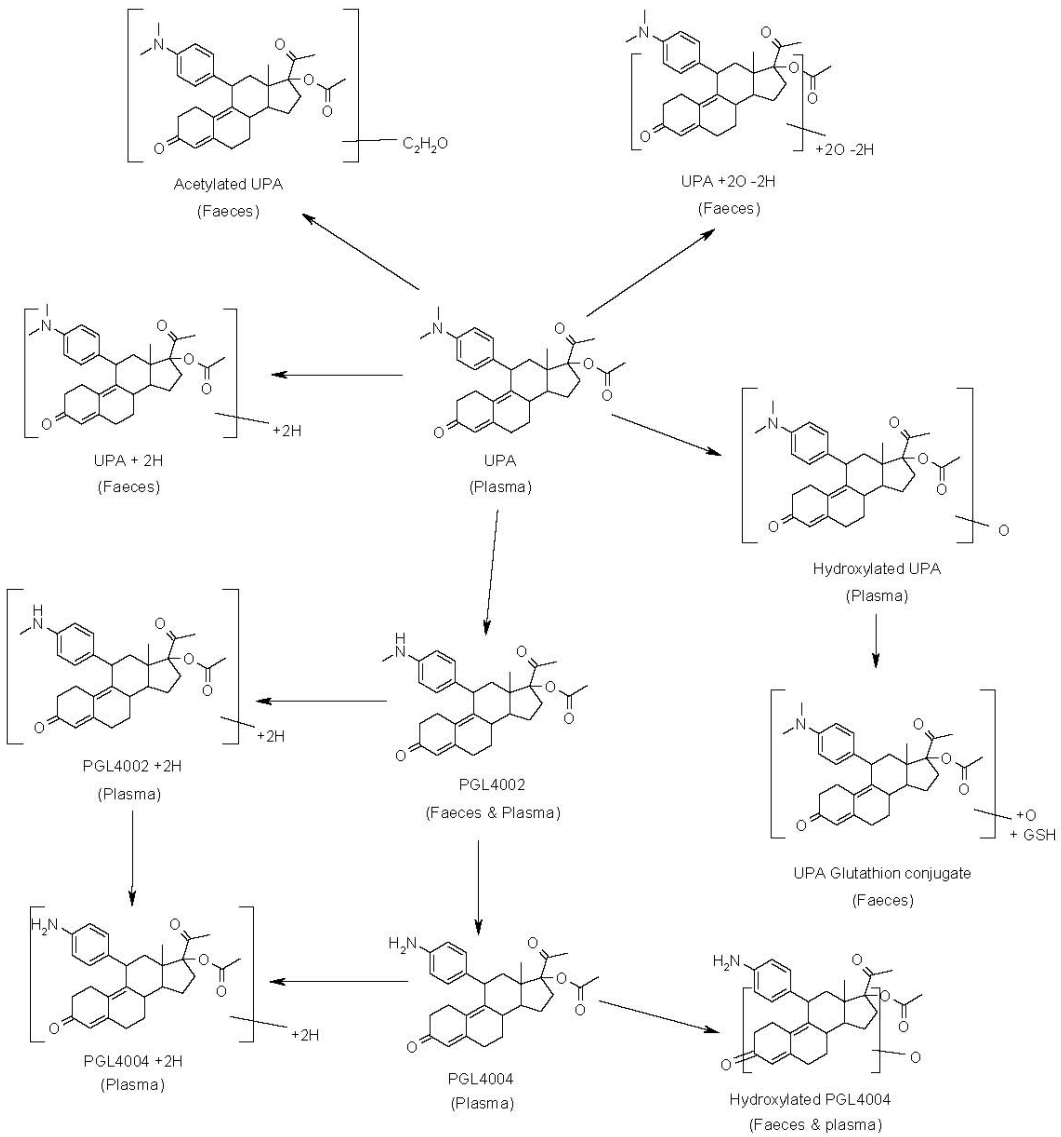

Figure 5: Proposed metabolic pathway for Ulipristal acetate (UPA).

Indeed, for mifepristone, the originator compound of this type of SPRMs, mono demethylated and didemethylated metabolites have also been identified in animals and humans following oral intake of mifepristone [16]. Jang and Benet [15] have demonstrated that the oxidation of mifepristone to its mono- and didemethylated metabolites has been catalysed by CYP3A4 [17]. As for UPA, a number of putative metabolites have been suggested by Attardi et al. [14] including PGL4002, PGL4004 and also 17α-hydroxylated UPA (ulipristal) or a UPA metabolite presenting an aromatic A-ring [18]. In this study, ulipristal was included as a standard, but could not be identified. However, a UPA + 2H metabolite was detected in the faeces, which could bear an aromatic A-ring.

UPA was extensively metabolized in premenopausal women

Approximately 73% of the radioactive UPA dose was recovered in the faeces within 11 days of administration of [^{14}C] UPA. Urine contained less than 7% of the radioactive dose. In faeces, metabolised UPA species, including a UPA glutathione conjugate, were the detected components; unchanged UPA was not identified. This may be due to the presence of intestinal CYP3A4 readily converting UPA in demethylated species.

The low levels of permissible radioactivity administered in this study limited classic metabolite profiling and identification and demanded alternative approaches to ensure the study objectives were met. With a moderate array of metabolites generated, the circulating concentrations of radioactivity in plasma were close to the limit of detection. To overcome this, plasma samples were pooled, extracted and concentrated approximately10-fold prior to radio-HPLC. Further concentration, or the use of increased sample volumes, could not improve chromatographic resolution due to proportional accumulation of endogenous material in the extracts. The potential for additional sample purification by Solid Phase Extraction was evaluated, but due to the preferential selectivity of this method for different metabolites, it was not deemed appropriate for use. The use of accurate mass detection allowed the interrogation of plasma and faeces for the presence of both radiolabelled and unlabelled UPA metabolites.

Conclusion

In conclusion, results of this study demonstrated that an oral dose of 20 mg of [^{14}C] UPA was well tolerated in premenopausal women. UPA was rapidly absorbed and extensively metabolized via successive demethylations of the 11β-dimethyl aminophenyl group via CYP3A4 oxidation. Levels of UPA and UPA-related radioactivity in blood and plasma declined slowly; there was a low level of blood cell binding of drug-derived radioactivity. The majority of the radioactive dose (73%) was excreted via the faeces. Renal elimination of total radioactivity was minimal, with less than 7% of the administered radioactivity being recovered in the urine.

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Competing Interests

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