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A 39-week oral toxicity study of ulipristal acetate in cynomolgus monkeys

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ABSTRACT

Ulipristal acetate (UPA) is a novel Progesterone Receptor Modulator (PRM) and registered for the pre-operative treatment of symptomatic uterine fibroids during 3 months. In a study which assessed the potential toxicity of UPA in female cynomolgus monkeys following daily oral administration of 1, 5, or 25 mg/kg for 39 weeks, UPA was well tolerated with dose-dependent macroscopic and microscopic observations limited to the uterus and oviducts. These findings were considered to be related to the pharmacological action of UPA and showed evidence of partial reversibility. Findings in the endometrium were similar to PRM-associated-endometrial-changes (PAEC) described in PRM-treated women. No adverse effects were found that would raise concerns about potential pre-malignancy. Although the translation of these findings to human is limited by the small study size and species differences, these results from animals chronically exposed to up to 150 times the clinical UPA exposure are considered significant and supportive to the chronic administration of UPA for more than 3 months in women of reproductive age.

1. Introduction

Progesterone plays a pivotal role in reproduction in many species. It is involved in the control of ovulation, implantation, and maintenance of pregnancy, and withdrawal of progesterone at the end of a non-fertile cycle results in menstruation in humans and nonhuman primates (Reel et al., 1979; Csapo and Pulkkinen, 1978). In the uterus, progesterone controls the growth and differentiation of endometrial and myometrial cells and directly regulates a variety of cell functions; it also acts indirectly by functionally opposing various estrogen effects. The recognition of the important role of progesterone in reproduction led to the development of synthetic progesterone receptor ligands, also known as Selective Progesterone Receptor Modulators (SPRMs). The synthesis of mifepristone (RU486), the first progesterone receptor antagonist, which also acted as a glucocorticoid receptor antagonist, was a starting point of drug discovery for progesterone receptor modulators throughout the world. Research focused on finding compounds with increased progesterone antagonistic potency and reduced antiglucocorticoid activity compared with mifepristone (Teutsch and Philibert, 1994; Spitz and Bardin, 1993; Bouchard et al., 2011). Ulipristal acetate (UPA) is a result of this search. This paper reports the design and results of a 39-week multiple dose study with an 8-week recovery period, conducted in cynomolgus monkeys. UPA is currently registered as a 3-month pre-operative treatment of uterine fibroids (ESMYA®), at a dose of 5 mg/day (PregLem, 2012). This long term regulatory toxicology study is part of the non-clinical safety program conducted to evaluate the potential toxicity of UPA for chronic use in women for more than 3 months. In the interest of animal welfare, this study was designed to use the fewest number of animals possible, consistent with the objective of the study, contemporary scientific standards, and in consideration of applicable regulatory requirements (ICH, 2009, 1998). In accordance with these requirements, the study size was sufficient for meaningful statistical analysis of data, identification of target organ toxicity and symptom recovery. Female cynomolgus monkeys were chosen as the animal model since the physiology of reproduction in this species is very similar to that in human (van Esch et al., 2008a,b). The cynomolgus undergoes a menstrual cycle of almost identical length (28–30 days) to the human (28–32 days), and the morphological appearances in endometrium of the normal follicular, luteal and menstrual phases show close similarities (van Esch et al., 2008a,b). As well as having close parallels in reproductive cycle physiology, macaques and humans also share a range of endometrial abnormalities of similar histological appearances, including endometrial polyps, hyperplasia and malignant neoplasms. For example, the endometrial response to short term unopposed estrogenic stimulation is similar in both species, with the development of simple non-atypical hyperplasia. In this context, Cline et al. (2008) noted that there is a tendency for regulators to regard the use of the term "endometrial hyperplasia"

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to represent complex hyperplasia with atypia, which may not be the case. Furthermore, these authors drew attention to important differences in the vocabulary of diagnostic terms used by veterinary and medical pathologists. The common human postmenopausal appearance of thickened endometrium with dilated but inactive glands is termed "cystic atrophy" by medical pathologists, but the similar appearance in macaques is described by veterinary pathologists as "cystic endometrial hyperplasia". We therefore considered it important that the endometrial changes on treatment were described in detail, rather than simply being given a diagnostic label.

In clinical studies, endometrium exposed to progesterone receptor modulators (PRMs) including UPA shows a range of histological changes referred to as PRM-associated endometrial changes (PAEC) (Mutter et al., 2008; Williams et al., 2007, 2012). The main histological findings of human PAEC are as follows: 1. Endometrial glands show architectural irregularity, and there is often extensive cystic dilatation. 2. Glandular epithelium appears inactive with low cuboidal, non-stratified epithelial cells showing infrequent mitoses. 3. There is a non-physiological secretory appearance, in which glands are coiled or tortuous (resembling those of the secretory phase), but with poorly developed secretory activity. 4. Glands are irregularly scattered throughout compact cellular stroma without pre-decidual change. Not all endometrial specimens exhibit every histological feature, but it is usually possible to recognize PRM-exposed endometrium by identifying several of the characteristic changes. It was of particular interest in this study of UPA-exposed monkey endometrium to determine whether an overall drug effect analogous to human PAEC could be recognized in treated animals, and whether effects were dose-related and reversible. It was also important to determine whether any of the morphological changes raised any concerns in terms of atypical or pre-malignant endometrial conditions.

2. Materials and methods

2.1. Test and control articles

UPA and control (Aqueous Suspending Vehicle (ASV): distilled water, sodium chloride, benzyl alcohol, polysorbate 80 (Tween® 80) and high viscosity carboxymethylcellulose) suspensions were each manufactured at least monthly at MPI Research (Mattawan, USA), and stored at 2–8 °C. Final dosing solutions were tested for stability and checked for concentration using a validated HPLC/UV method. The analytical results verified that the solutions had been prepared properly and were stable under the conditions of use.

2.2. Test animals and husbandry

Cynomolgus monkeys were supplied by Harlan Laboratories (Blue Mounds, WI, USA) and acclimatized to laboratory conditions for 49 days prior to receiving the first dose of test or control article. Mature female monkeys were used in this study (40–61 months at receipt) weighing between 2.2 and 3.1 kg. The monkeys were kept in steel cages (one animal per cage) with the room temperature maintained at 18–29 °C. Food was offered to the monkeys twice a day and water was provided ad libitum. This study was conducted at MPI Research (Mattawan, MI, USA) in compliance with current US FDA Good Laboratory Practice Regulations for Non-Clinical Laboratory Studies (21 CFR Part 58) and the Organization for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17). The study protocol complied with the USDA Animal Welfare Act (9 CFR Parts 1, 2 and 3). The Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Academy Press, Washington, D.C., 1996, was followed.

2.3. Study design

The study consisted of four groups, each comprising four female cynomolgus monkeys. The groups either received ASV (control), or UPA at dose levels of 1, 5, or 25 mg/kg for 39 weeks. Two additional animals were allocated to the control and high dose groups for an 8-week post-dose recovery period. At randomization, there was no statistically significant difference between treatment groups in mean body weight. The vehicle or UPA was administered to all groups by oral gavage for 273 consecutive days at a dose volume of 2 mL/kg. Following the dosing or recovery period, animals were euthanized by intravenous administration of sodium pentobarbital followed by exsanguination of the femoral vessels.

2.4. Observations

Observations and measurements were conducted according to the schedule shown in Table 1. All animals were also evaluated for vaginal bleeding (menses) using a vaginal swabbing procedure.

2.5. Exposure control and bioanalytical assessments

Blood samples for the determination of UPA and its *N*-monodemethylated metabolite PGL4002 were collected from the femoral vein into tubes containing lithium heparin according to the schedule in Table 1. Samples were prepared and analyzed for UPA and PGL4002 using a validated LC-MS/MS assay (MPI Research, State College, PA, USA; data on file). Over the calibration range 1–500 ng/mL, the overall imprecision and inaccuracy of the assay were 2.9–5.5% and 0.0–1.2% for UPA, respectively. Over the same calibration range, overall imprecision and inaccuracy of the PGL4002 assay were 2.5–6.7% and 0.0–1.6%, respectively. Incurred sample reanalysis was also performed successfully for parent and metabolite.

Table 1
Schedule of animal observations/measurements.

Procedure	Frequency of evaluation
Cageside observations	⩾2 per day; morbidity, mortality, injury, and the availability of food and water
Detailed clinical observations	Weekly; evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and bizarre behavior
Determination of menses and menstrual cycles	Daily; qualitative assessment: none, slight, moderate, severe
Body weights	Weekly
Ophthalmoscopic examinations	Pretest and prior to the terminal and recovery necropsy
Electrocardiographic examinations	Pretest and at 1–2 h post-dose on day 1 and once during week 13, 26, and 39 and once during the last week of the recovery period
Clinical pathology	Pretest, once during weeks 4, 13, and 26, and prior to the terminal and recovery necropsies
Exposure control, cortisol and prolactin assessments	Daily from days -3 to -1, and at approximately 4 h post-dose daily from days 1–8, once weekly from weeks 3–13, approximately monthly from months 4–9, and approximately monthly during the recovery period