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A Six-Month Baboon Study Comparing Two Anti-Hormones Given Separately and Simultaneously

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ABSTRACT

Background: *Telapristone (CDB-4124 or Proellex®) is a PRM (progesterone antagonist) under development for treatment of uterine fibroids and endometriosis in women. Enclomiphene citrate (Androxal®, EnCyzix®) is a SERM (estrogen antagonist) under development for testosterone restoration in men with secondary hypogonadism. Telapristone exhibits potent anti-proliferative effects on the endometrium. In human studies longer than four months a dose-dependent suppression of endometrial thickening was observed by ultrasound and confirmed by tissue biopsy. As the dose of the drug was increased there was an increase of anti-proliferative and pro-apoptotic events. Enclomiphene citrate has been administered to rodents, dogs, baboons and humans and elevates total serum testosterone levels in male baboons and humans. In a study evaluating the effects of clomiphene and its isomers, enclomiphene (trans-Clomiphene citrate) and zuclomiphene (cis-Clomiphene citrate), in nine male baboons, Enclomiphene citrate was safely administered with maximal effects on serum testosterone at an oral dose of 1.5 mg/kg of body weight for 12 days. We have shown that the effects are driven by its central anti-estrogenic action. Administration to men has demonstrated no decreases in bone mineral density measured by DEXA, a consideration for any SERM. This study investigated combinations of anti-hormones for the treatment of cancers of the female reproductive system, (i.e., breast, uterine, and ovarian) and was driven by Proellex's anti-proliferative effects. Dual administration with Androxal has the potential of preserving bone parameters while providing an estrogen antagonist.*

Methods: *Baboons were used to approximate findings in human subjects due to the relatedness of the reproductive system and cycle. Three cycling females per group were treated for six months with appropriate doses of Proellex, Androxal or a combination of the two. Cycling, physical signs, hormones, uterine ultrasounds, DEXA analysis and clinical chemistry were assessed.*

Results: *Proellex did not disrupt the tumescence index, an estrogen dependent index of female baboons. Enclomiphene completely stopped the index. Both Proellex and Androxal, alone and in combination, stopped vaginal bleeding, an indication of cycle disruption, perhaps centrally. One unusual finding was that Enclomiphene induced development of a distinct color of the baboon sex skin. This color is indicative of pregnancy, but chorionic gonadotropins were negative. Blood chemistries, compared to controls, were unchanged in all groups. DEXA analysis demonstrated no changes in body composition. No changes in uterine and ovarian pathology were seen compared to controls.*

Conclusion: *Administration of Proellex and Androxal were shown to have disruptive effects on baboon reproductive cycling while producing few other non-hormonal detrimental effects.*

Keywords: Clomiphene, Enclomiphene, Proellex, Telapristone, Baboon

INTRODUCTION

Studies comparing dual administration of Androxal, an anti-estrogen, and Proellex, an anti-progestin, structures seen in Figure 1, have not been performed in this species. The use of baboons will approximate finding expected to result in human subjects due to the close relatedness of the reproductive system and similar cycles. Baboons are advantageous in this study also because of the external sex skin which will allow monitoring of the reproductive cycle during the course of this study [1-6]. This study was developed to investigate Proellex in development of future treatments for cancers of the female reproductive system, (i.e., breast cancer, uterine cancer and ovarian cancer) due to Proellex's anti-proliferative effects. Dual administration with Androxal has the potential of increasing bone parameters seen with administration of current anti-progestin treatments of reproductive cancers.

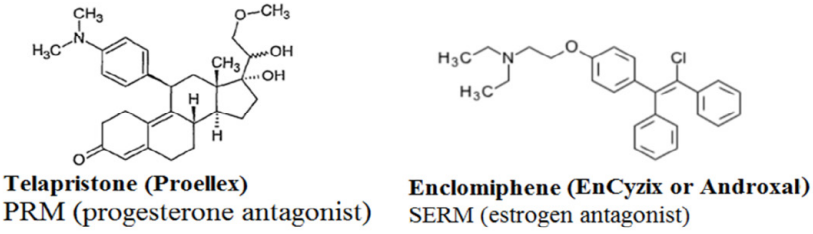

Figure 1: Structure of proellex and enclomiphene

Proellex acts against progesterone and has been shown to exhibit potent anti-progesterone effects [7-9]. Acute toxicity studies in mice suggest that the acute oral LD50 is greater than 1250 mg/kg. A daily dosing of Proellex at 50 mg/kg of body weight for a period of four weeks in mice showed that this treatment produced no adverse effects.

In studies longer than four months, in humans, a dose dependent suppression of endometrial thickening was observed and later confirmed by assessment of tissue obtained by biopsy from women exposed to Proellex for an extended period of time. As the dose of the drug is increased there is an increase of anti-proliferative and pro-apoptotic events. Recommendations by the FDA have restricted the dose of Proellex to 12 mg, or below, due to elevated liver enzymes observed in 3.5% women dosed with 50 mg Proellex (internal results of clinical trials).

Enclomiphene citrate (Androxal) has been safely administered to rodent, canine, baboon species, as well as humans, and has been shown to elevate total serum testosterone levels in canines, baboons and men, but not rats, suggesting interspecies differences exist in the pharmacologic response to Enclomiphene citrate.

Enclomiphene is one of two isomers of Clomiphine. Clomiphene citrate has been approved for use in women to induce ovulation [10-13] and has been used off-label in men to raise serum testosterone [14-19]. The latter use was rationalized early [20] by the recognition that a bolus dose of Clomiphene citrate could be used as a diagnostic tool to determine the functionality of the hypothalamic-pituitary axis by the stimulation of release of Luteinizing Hormone (LH) in a subject with low gonadotropins.

Clomiphene citrate is considered to be a SERM, i.e., a compound possessing estrogen agonist or antagonist properties depending on the tissue. Clomiphene citrate was described as a mixture of two isoforms, enclomiphene (trans-isomer) and zuclomiphene (cis-isomer), with estrogen agonist or antagonist properties [21-24].

Enclomiphene citrate has anti-estrogenic properties [25] and appears to block the negative feedback inhibitory effects of estradiol on the hypothalamic-pituitary axis resulting in increased levels in both LH and follicle stimulating hormone (FSH) which stimulate endogenous intragonadal testosterone production and spermatogenesis in men [17,26].

Evaluating the effects of clomiphene, enclomiphene and zuclomiphene in male baboons (unpublished results), enclomiphene was safely administered in an oral dose of 1.5 mg/kg of body weight for 12 days to 3 male baboons. Administration in men has demonstrated increases in bone parameters measured by DEXA.

MATERIALS AND METHODS

Descriptions of groups

This study compared two different compounds that are under clinical development by Repros Therapeutics, The Woodlands, TX. Enclomiphene citrate (Androxal®, Encyzix®, is an anti-estrogen underdevelopment for testosterone replacement in men with secondary hypogonadism and Proellex® (CDB-4124, Telapristone), an anti-progestin under development for treatment of uterine fibroids. Four treatment groups were included in the study. Group 1 Placebo control; Group 2 Proellex (12 mg/day); Group 3 Androxal (25 mg/day); and Group 4 Combination of the two Compounds (Proellex 12 mg and Androxal25 mg).

The study was performed at Texas Biomedical Research Institute in San Antonio Texas. Normal, cycling female baboons (*Papiohamadryas*) of prime reproductive age were used in the study. All groups underwent identical manipulations. Each group was administered compound daily for 6 months. After 6 months, dosing was stopped and animals observed until commencement of normal vaginal bleeding began. Procedures are described as follows.

Animal acquisition and acclimation

Veterinary Services performs an evaluation of each animal for potential enrollment in a study, which included:

1. Physical examination, complete blood count (CBC), and chemistry panel.

2. Review of an animal's social and behavioural data, clinical history and research history.

3. Further diagnostics as specified by protocol.

Test article administration

Justification for route of administration

The oral route was the intended route of administration of these test articles in humans. The vehicle control and test articles were administered once per day for 6 months orally. Compounds were emptied from the gelatin capsules and added to a cup of flavoured drink(e.g. kool-aid, sugar-free juice, tang, Gatorade) or placed inside a medium for oral dosing (e.g. Banana, cookie dough, buck eye cookies, orange, apple sauce, peanut butter and jelly sandwich).

Cage side observations

All animals were observed every day by qualified technicians. Body weights for all animals were measured and recorded at months 0-7 during the study. Results are included in DEXA results. Daily observations were made for Tumescence (external sex skin swelling, 0=no swelling to 4=maximum swelling), vaginal bleeding and external sex skin colour changes.

Clinical chemistries

Hematology evaluations were conducted on all animals pre-test and during Months 0-7. Blood samples (approximately 4 to 6 mL) were collected from the jugular vein. No anticoagulant was used for the clinical chemistry samples. Samples were evaluated on the UniCelD × C 600 Clinical System (Beckman Coulter).

Blood drug level analysis

Blood samples with K_2-EDTA (approximately 2 mL) were collected for analysis of blood level concentrations of test materials and major metabolite. Plasma samples collected were shipped at the end of the study to BASi (West Lafayette, IN) for analysis. Compounds evaluated were CDB-4124 (Proellex), CDB-4453 (mono-demethylated metabolite of CDB-4124), enclomiphene (Androxal), and 4-hydroxy enclomiphene (primary metabolite of enclomiphene).

Hormone analysis

Blood samples (approximately 2 mL) were collected on all animals pretest and Months 1-7 (once per month),via the jugular vein, for hormone evaluations of estradiol (E2), luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone (P4), testosterone (T) and hCG. The blood samples were shipped to Antech Diagnostics (Morris ville,North Carolina) for analysis.

DEXA analysis

Animal were sedated at pretest and Month 6 with Ketamine HCl IM (10 mg/kg), weighed and brought to the DEXA room. The animal was measured and was placed in dorsal recumbency on the DEXA table. A General Electric iDexa machine was used for the procedure. The total body scan began approximately 2 cm above the animals head and continued through the bottom of the animal's feet taking in the entire body.

Uterine/ovarian ultrasounds

Animals were sedated at pre-test and Month 6 with Ketamine HCl (10 mg/kg) and brought to the ultrasound room. The animal was placed in dorsal recumbency, hair removed from abdomen, and ultrasound gel was liberally applied to the area. A General Electric Health Care LOGIQ 5 machine with a 5-8 MHz transducer was used for the procedure. The transducer was placed just rostral to the pubis and the uterus was identified by the endometrial stripe. Moving rostrally, the ovaries were identified as oval structures just lateral to the uterus, almost to the most cranial portion of the uterus. Images were captured of both ovaries and the uterus before the animal was moved to the surgery suite for the endometrial biopsy.

Uterine biopsies

Baboons were sedated at pretest, Month 6 and at the resumption of cycling after discontinuation of treatment with an intramuscular dose of Ketamine HCl (10 mg/kg), intubated, and maintained on Isoflurane 1-3%. The animals were placed in a prone position, with their legs flexed under their body, and the tail retracted cranially to give maximum exposure to the vulva. A lubricated vaginal speculum was inserted into the vagina to allow visualization of the cervical opening. Stainless steel cervical dilators were inserted thru the cervical opening to dilate and allow easy passage of the biopsy instrument. A Uterine Explora™ Curette was introduced thru the cervix into the uterus. Once in the uterus, the stylet was removed and the Vacu-Lok Syringe attached. The syringe plunger was withdrawn to the 12 cc mark and locked in place to provide enough vacuum to pull endometrial lining into the curette tip. The curette was then withdrawn slightly but remained in the uterus, reinserted, rotated about 90 degrees and withdrawn again. When sufficient tissue was obtained, the syringe lock was disengaged, the vacuum released, and the curette removed. Contents of the curette were collected in a conical tube with 10% neutral buffered formalin.

RESULTS

Observations were made on individual animals to determine vaginal bleeding and Tumescence index (degree of sex skin swelling). And sex skin development of pregnancy color. Results of these observations are presented in Figure 2.

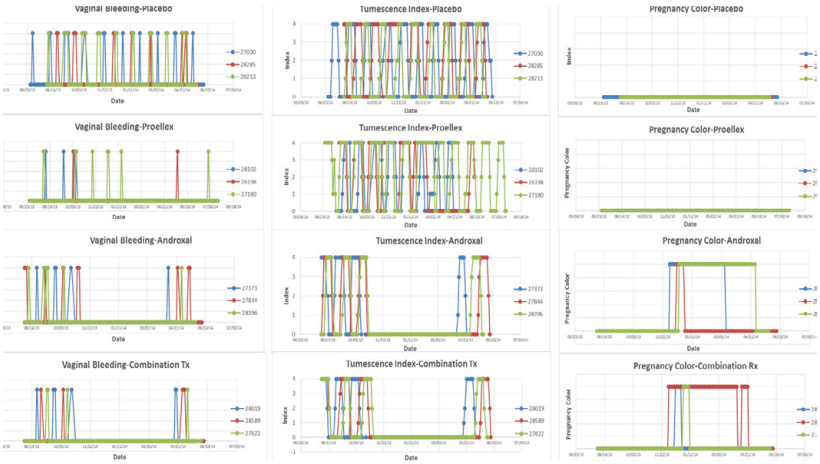

Figure 2: Daily observations

Vaginal bleeding stopped in Group 2, 3 and 4 during drug administration. Tumescence index (swelling of the external sex skin) was graded on a scale of 0 (no swelling) to 4 (maximum swelling). Animals in Group 1 (Placebo) and Group 2 (Proellex) displayed normal patterns of tumescence. For animals in Group 3 (Androxal) and Group 4 (Proellex and Androxal) all swelling stopped during drug administration. An unexpected, and unexplained, result was the development of a sex skin coloration that was usually associated with pregnancy in animals administered Androxal (Groups 3/4). Blood concentrations of test materials and metabolites are shown in Figure 3.

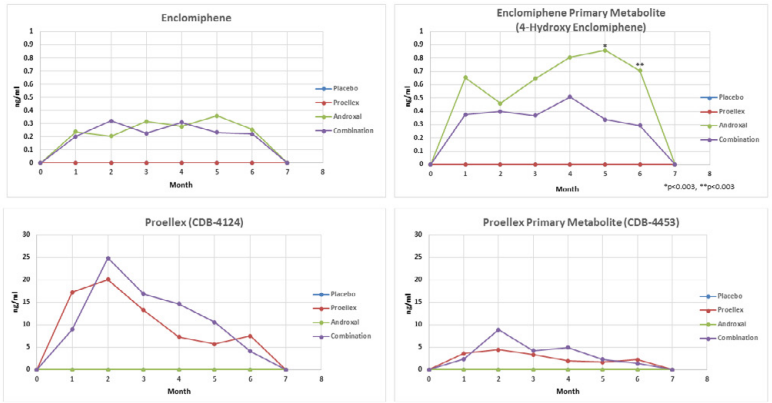

Figure 3: Blood levels of parent compound and metabolites

All compounds administered were above detectable limits including the primary metabolites that were analyzed in this study. Hormonal analysis is presented in Figure 4.

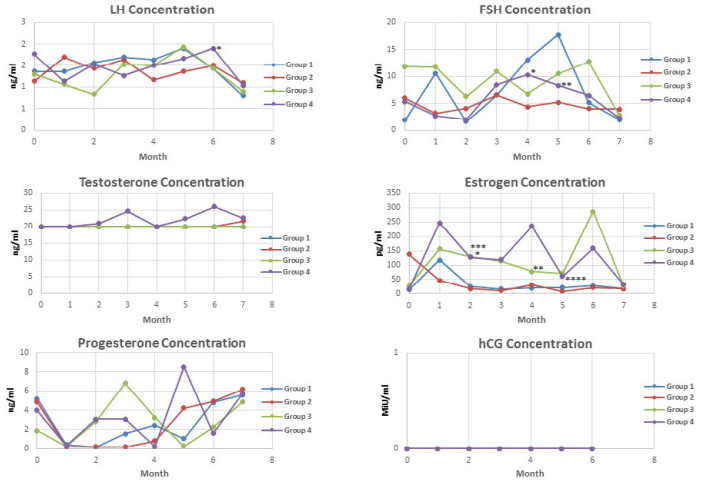

Figure 4: Hormone levels

Results of DEXA analysis to determine changes in body composition are presented in Table 1.

Table 1: DEXA analysis: body composition

ID	Group	Drug	Date	Age (Years)	Tissue (g)	% change from Baseline	Fat(g)	% change from Baseline	Lean (g)	% change from Baseline	BMC (g)	% change from Baseline	Fat Free (g)	% change from Baseline
28213	1	Placebo	4/17/2014	8.2	16317	11.06	3049	38.21	13268	6.25	784.4	-2.28	14052	5.74
28285	1	Placebo	4/18/2014	7.6	12935	-11.54	1115	-35.70	11820	-8.29	753.5	-1.90	12574	-7.92
27030	1	Placebo	3/24/2014	8.5	15939	-22.64	1495	-70.55	14444	-6.99	780.1	-6.41	15224	-6.96
Mean				8.10	15063.7		1886.33		13177.33		772.67		13950.00	
SD				0.46	1853.14		1024.67		1314.35		16.74		1327.94	
P<				0.301	0.523		0.404		0.727		0.247			
ID	**Group**	**Drug**	**Date**	**Age (Years)**	**Tissue (g)**	**% change from Baseline**	**Fat(g)**		**Lean (g)**	**% change from Baseline**	**BMC (g)**	**% change from Baseline**	**Fat Free (g)**	
26198	2	Proellex	4/16/2014	9.1	18553	0.41	3993	-20.90	14560	8.43	832.7	-4.03	15393	7.67
27180	2	Proellex	7/11/2014	8.8	18351	-7.44	2758	-20.01	15593	-4.79	800.9	-3.44	16394	-4.72
28012	2	Proellex	3/24/2014	7.7	13052	-9.39	1265	-18.33	11787	-8.32	735.7	-4.23	12523	-8.09
Mean				8.53	16652.0		2672.00		13980.00		789.77		14770.00	
SD				0.74	3119.33		1366.03		1968.17		49.45		2009.29	
P<				0.507	0.725		0.626		0.886		0.475			
ID	**Group**	**Drug**	**Date**	**Age (Years)**	**Tissue (g)**	**% change from Baseline**	**Fat(g)**		**Lean (g)**	**% change from Baseline**	**BMC (g)**	**% change from Baseline**	**Fat Free (g)**	
27844	3	Androxal	3/24/2014	7.7	13052	-9.39	1265	-18.33	11787	-8.32	135.7	-82.34	12523	-8.09
27373	3	Androxal	4/1/2014	8.4	12956	-1.64	1503	13.78	11453	-3.36	608.9	-8.68	12062	-3.64
28396	3	Androxal	4/17/2014	7.5	17895	-4.04	4278	-7.14	13617	-3.01	699.8	-7.34	14317	-3.23
Mean				7.87	14634.3		2348.67		12285.67		481.47		12967.33	
SD				0.47	2824.23		1675.08		1165.00		302.87		1191.36	
P<				0.287	0.756		0.925		0.533		0.234			
ID	**Group**	**Drug**	**Date**	**Age (Years)**	**Tissue (g)**	**% change from Baseline**	**Fat(g)**		**Lean (g)**	**% change from Baseline**	**BMC (g)**	**% change from Baseline**	**Fat Free (g)**	

27622	4	Combi-nation	4/18/2014	8.22	18092	1.17	1812	-19.47	16279	4.14	807.5	-5.79	17086	3.62
28019	4	Combi-nation	3/27/2014	7.8	17722	3.97	2889	10.27	14833	2.82	685.4	-7.25	15519	2.33
28589	4	Combi-nation	4/17/2014	7.3	15302	-4.12	1936	26.04	13366	-7.33	782.4	-5.63	14148	-7.24
Mean				7.77	17038.7		2212.33		14826.00		758.43		15584.33	
SD				0.46	1515.33		589.28		1456.51		64.48		1470.09	
P<				0.276	0.945		0.877		0.999		0.387		0.960	

Parameters measured were Tissue (g), Fat (g), Lean (g), Body Mass composition (BMC) and Fat Free (g). Changes per animal are presented with a change in baseline. Statistics presented were determined by Student t-test analysis.

Examination of the uterus and ovaries by ultrasound techniques are presented in Table 2 and uterine biopsies in Table 3.

Table 2: Uterine and ovarian ultrasounds

					Uterus (cm)		Left Ovarv (cm)		Right Ovarv (cm)			Uterus (cm)		Left Ovarv (cm)		Right Ovarv (cm)	
Tx	**Animal ID**	**Start Dosing**	**Final Dose**	**Baseline Ultra-sound**	**1L**	**2L**	**1L**	**2L**	**1L**	**2L**	**Mth6 Ultrasound**	**1L**	**2L**	**1L**	**2L**	**1L**	**2L**
1	27030	10/4/13	3/23/14	10/3/13	1.51	2.51	1.26	1.67	1.39	1.74	3/24/14	1.44	2.48	1.14	1.6	1.15	1.08
1	28285	10/16/13	3/31/14	10/15/13	1.83	3.08	1.33	1.11	0.99	1.52	4/18/14	1.85	1.97	0.9	128	0.99	1.52
1	28213	11/1/13	4/16/14	10/31/13	1.57	3.6	1.06	1.32	1.54	1.43	4/17/14	1.2	1.36	0.95	0.83	0.68	0.93
				Mean	1.64	3.06	1.22	1.37	1.31	1.56	Mean	1.50	1.94	1.00	1.24	0.94	1.18
				SD	0.17	0.55	0.14	0.28	0.28	0.16	SD	0.33	0.56	0.13	0.39	024	0.31
										BLvs 6Mt	P <	0.548	0.067	0.114	0.663	0.162	0.125
2	28102	10/4/13	3/23/14	10/3/13	1.6	3.01	0.86	1.4	0.82	1.27	3/24/13	1.39	3.02	0.85	1.25	1.07	127
2	26198	10/31/13	4/15/14	10/30 13	1.81	3.38	0.76	0.93	0.66	1.03	4/16/14	2.88	2.92	1.68	2.56	1.57	2.43
2	27180	1/17/14	7/11/14	1/16/14	0.87	2.11	0.8	1.22	0.74	1.01	7/11/14	1.46	1.6	122	0.5	0.78	0.93
				Mean	1.43	2.83	0.81	1.18	0.74	1.10	Mean	1.91	2.51	125	1.44	1.14	1.54
				SD	0.49	0.65	0.05	024	0.08	0.14	SD	0.84	0.79	0.42	1.04	0.40	0.79
										BLvs 6Mt	P <	0.439	0.618	0.141	0.703	0.164	0.395
3	27373	10/16/13	3/31/14	10/15/13	2.72	3.14	NA	NA	NA	NA	4/1/14	2.36	2.25	1.85	2.16	2.08	2.21
3	27844	10/31/13	4/15/14	10/30/13	1.89	3.06	0.58	0.85	0.76	1.19	4/16/14	1.84	1.71	NA	NA	NA	NA
3	28396	11/1/13	4/16/14	10/31/13	1.44	2.62	0.61	1.35	0.77	1.41	4/17/14	1.53	1.71	0.66	1.03	1.17	0.87
				Mean	2.02	2.94	0.60	1.10	0.77	1.30	Mean	1.91	1.89	1.26	1.60	1.63	1.54
				SD	0.65	0.28	0.02	0.35	0.01	0.16	SD	0.42	0.31	0.84	0.80	0.64	0.95
										BLvs 6Mt	P <	0.823	0.012	0.383	0.507	0.199	0.758
4	28019	10/11/13	3/26/14	10/10 13	1.73	1.66	1.07	1.61	0.68	1.71	3/27/13	1.44	2.7	0.84	1.13	125	1.55
4	27622	11/2/13	4/17/14	11/1/13	1.46	3.28	0.62	0.76	0.74	0.91	4/18/14	0.91	1.92	1.48	1.85	0.89	1.59
4	28589	11/1/13	4/16/14	10/31/13	2.57	3.78	1.17	1.41	1.43	1.6	4/17/14	2.1	1.5	1.36	0.91	1.19	0.7
				Mean	1.92	2.91	0.95	1.26	0.95	1.41	Mean	1.48	2.W	123	1.30	1.11	1.28
				SD	0.58	1.11	0.29	0.44	0.42	0.43	SD	0.60	0.61	0.34	0.49	0.19	0.50
										BLvs 6Mt	£<	0.414	0.301	0.351	0.928	0.579	0.758

Table 3: Uterine biopsies

Tx	**Animal ID**	**First Dose**	**Final Dose**	**Baseline**	**Diagnosis**	**Month 6**	**Diagnosis**	**Post-Cycle**	**Diagnosis**

1	27030	10/4/13	3/23/14	10/3/13	Essentially normal tssue uterus. PCH	3/24/14	Metritis, supportive, multifocal, min-mild, uterus PCH	5/5/14	Essentially normal tissue, uterus. PCH
1	28285	10/16/13	3/31/14	10/15/13	Non-diagnosis, PCX	4/18/14	Non-diagnostic sample, PCX	5/22/14	Normal, uterus, PCA
1	28213	11/1/13	4/16/14	10/31/13	Normal, endometrium, PCX	4/17/14	Normal, uterus. PCX	5/19/14	Normal, uterus. PCX
2	28102	10/4/13	3/23/14	10/3/13	Essentially normal tissue, uterus. PCH	3/24/14	Essentially normal tissue, uterus. PCA	4/21/14	Normal, uterus, PCA
2	26198	10/31/13	4/15/14	10/31/13	Normal, endometrium, PCA	4/16/14	Normal, uterus. PCA	6/8/14	Fibrosis, multifocal, mild, uterus. PCA
2	27180	1/17/14	7/11/14	1/16/14	Normal, uterus, HAN	7/11/14	Normal, uterus, HAN	8/15/14	Essentially normal tissue, Uterus. HAN
3	27373	10/16/13	3/31/14	11/6/13	Cervicitis, supportive, min-mild. cervix	4/23/14	Non-diagnostic sample, PGO	NE	
3	27844	10/31/13	4/15/14	10/30/13	Normal, endometrium, PCA	NE		6/5/14	Normal, uterus. PCA
3	28396	11/1/13	4/16/14	10/31/13	Normal, endometrium, PCA	4/17/14	Metritis, lymphocytic, diffuse, minimal, uterus, PCA	5/22/14	Normal, uterus
4	28019	10/11/13	3/26/14	10/10/13	Essentially normal tissue, uterus. PCA	3/27/14	Non-diagnostic sample, uterus. PCA	5/9/14	Normal, uterus, PCA
4	27622	11/2/13	4/17/14	11/1/13	Normal, adipose tissue, PCA	4/18/14	Non-diagnostic sample, PCX	5/29/14	Non-diagnostic, uterus, PCX
4	28589	11/1/13	4/16/14	10/31/13	Normal endometrium, PCX	4/17/14	Non-diagnostic sample	6/5/14	Normal, uterus. PCA

DISCUSSION

Observations made to determine effects of compound administration on vaginal bleeding showed the expected result of administration of Proellex, an anti-progestin, (Group 2 and 4). Bleeding ceased and the uterus became quiescent. These results had been observed previously in human clinical trials. Removal of the action of progesterone should produce a quiescent uterus and stop normal cycling. The administration of Enclomiphene citrate, an anti-estrogen, has not been performed previously in humans or baboons. The appearance of a quiescent uterus after Enclomiphene citrate administration was equal to that seen by Proellex. Tumescence index (swelling of the external sex skin) was graded on a scale of 0 (no swelling) to 4 (maximum swelling). Proellex administered animals displayed normal patterns of tumescence. For animals in which Androxal was administered (Groups 3 and 4) Tumescence was measured as 0. After drug administration was discontinued the vaginal bleeding and tumescence index returned, indicating normal cycling commencement, after approximately 4 weeks.

An unexpected result was the development of a coloration of the sex skin indicative of pregnancy. Animals administered Androxal, an anti-estrogen, (Group 3/4) developed, at some point during drug administration and for differing durations, a specific color of the external sex skin that is normally, in baboons, an indication of pregnancy. No explanation can be offered for this development at this time. Animals were examined by multiple methods to determine pregnancy status. First, animals were singly housed and were examined for pregnancy prior to inclusion in the study. No fetal development was seen in any animal during uterine ultrasound procedures. Hormonal analysis for human chorionic gonadotropin (hCH) was negative at all months for all animals. The development of the unexpected pregnancy color remains unexplained, but was not related to pregnancy in this study. After discontinuation of treatments the pregnancy indicator color was not observed.

Testing determined all compounds administered and their primary metabolites achieved levels detectable in blood. Each compound achieved similar blood concentrations of parent or metabolite whether administered alone or in combination with the exception 4-Hydroxyenclomiphene. Co-administration of Proellex with Androxal resulted in lower blood levels of 4-Hydroxyenclomiphene compared to levels achieved when Androxal was administered alone (significant by Student's t-test, 5 and 6 months, $p<0.003$). This difference of metabolism when the two compounds are administered simultaneously will be further analyzed in future clinical trials.

There were significant differences in hormonal levels at several months of treatment, but no consistent rises or falls were seen with any treatment. Estrogen levels trended higher in Group 3 (Androxal) at with a significant difference

when compared to controls at Month 2 (*p<0.005) and Month 5 (**p<0.05) and in Group 4 (Proellex and Androxal) at Month 2 (***p<0.03) and Month 3 (****p<0.03). LH was seen to be elevated in Group 4 at Month 6 (p<0.01). Sustained elevation of LH, normally a transient elevation, due to negative feedback of elevated estrogen may account for the observation of the pregnancy color indicator of the external sex skin. To date, this is our only explanation of the observation. Human chorionic gonadotropic hormone was not detected in any of the groups at any time point. This confirms along with other methods used, that the animals were not pregnant. FSH was significantly elevated in Group 4 at Month 4(*p<8.5×10^{-10}) and Month 5 (**p<8.5×10^{-6}). The irregular elevations seen between animals could be due to the small number of animals that were examined in this study. Another factor to consider when evaluating the hormonal status of the animals was that samples were only acquired once per month in the study and not a daily collection.

No statistically significant differences by DEXA examination (body composition, fat distribution and body mass composition) were found in any of the groups when compared to placebo controls.

No significant differences in measurements of the uterus or ovary were seen except for the mean decrease in size of the uterus of Group 3 animals. This was not confirmed in animals dosed with both compounds.

Examination of the uterus and ovaries by ultrasound did not identify any differences between baseline samples and samples collected after the resumption of cycling after discontinuation of treatments. All samples collected at the six month collection in the combination group were described as Non-Diagnostic samples. During collection of samples it was noted that samples were difficult to obtain. Whether this was a pathological effect of the combination treatment cannot be determined. As noted above, ultrasound examination of the uterus did not reveal any abnormalities.

CONCLUSION

Administration of Androxal or Proellex, either alone or in combination resulted in few abnormalities. Disruptions in the reproductive cycle were the primary defects seen. Both Androxal and Proellex disrupted vaginal bleeding while only Androxal, both alone and in combination with Proellex, disrupted the tumescence index (sex skin swelling) of treated animals. The swelling of the external sex skin of baboons is under the influence of estrogen. The anti-estrogenic effects of Androxal was most likely the cause of the lack of cycling of the swelling pattern of the external sex skin. Androxal, alone and in combination with Proellex, also caused the appearance of pregnancy color of the sex skin. The color change indicating pregnancy in normal baboons might be due to sustained levels of LH, a condition not normally seen in primates. Measurements of hCG and uterine ultrasounds confirmed the absence of pregnancy.

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