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Ulipristal acetate administration at mid-cycle changes gene expression profiling of endometrial biopsies taken during the receptive period of the human menstrual cycle

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ABSTRACT

The aim of this study was to analyze the effects of mid-cycle administration of Ulipristal acetate (UPA) on gene expression in endometrial biopsies taken during the receptive phase of the cycle. Fourteen healthy menstruating women were studied during 14 control non-treated and 12 treated cycles with a single dose of 30 mg UPA when follicle diameter reached 20 mm. Ovulation in both treated and control cycles was confirmed by serial determinations of serum LH, progesterone and vaginal ultrasound. An endometrial biopsy at day LH+7, in each cycle, was taken for RNA microarray and qPCR analysis or prepared for histological and immunohistochemistry studies. Functional analysis of differentially expressed genes showed the presence of changes compatible with a non-receptive endometrial phenotype, further confirmed by qPCR and immunohistochemistry. This study suggests the effects of UPA on endometrial receptivity, offering a plausible explanation for the higher contraceptive efficacy of this method compared to that of levonorgestrel.

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1. Introduction

The search for new contraceptive regimens aimed at finding more effective methods with no side effects is still an ongoing issue in contraceptive research. Levonorgestrel (LNG), a synthetic 19-nor testosterone derivative, is widely used as an oral effective and safe emergency contraceptive (EC). LNG interferes with ovulation if taken prior to the occurrence of the LH peak, but not when given at the time or after the mid-cycle pituitary LH surge (Croxatto et al., 2004; Durand et al., 2001; Hapangama et al., 2001). This condition might explain why the effectiveness of LNG as EC in terms of pregnancy prevention declines with treatment delay. Among the relative recent drugs developed, mifepristone, a progesterone receptor modulator, is effective for both pregnancy termination and EC (Croxatto, 2003). In addition, a set of compounds with 11β-aryl substitutions has been synthetized with effective progesterone receptor (PR) antagonist activity (Attardi et al., 2002; Chabbert-Buffet et al., 2005; Reel et al., 1998). From all of them, only Ulipristal acetate (UPA), a 19-nor progesterone derivative, has been licensed for clinical use. Ulipristal is an orally active selective progesterone receptor modulator with tissue-selective antagonist activity (Blithe et al., 2003; Cook et al., 1994). As in the case of LNG, UPA effects on follicular rupture (FR) depend on the time of its administration in the menstrual cycle and both of them cannot prevent ovulation when given after the mechanisms of LH surge at mid-cycle have been initiated. Ulipristal is as effective contraceptive as LNG when given up to 72 h after coitus, but is highly superior to LNG when administered even after 120 h of unprotected intercourse (Brache et al., 2010, 2013). These results agreed with other studies comparing the contraceptive efficacy of UPA with that of

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LNG (Creinin et al., 2006; Glasier et al., 2010). This evidence suggests that efficacy of UPA over that of LNG might rely on other mechanisms than those concerning with delaying or inhibiting ovulation, especially because ovulation does not occur when UPA is given at the time of LH peak (Brache et al., 2010). These observations, together with those published on the effects of UPA on endometrial progesterone-dependent markers (Stratton et al., 2000, 2010), indicate that part of its contraceptive effects, particularly in those women remaining ovulatory after treatment, might involve the endometrium. This statement contrasts to that of international scientific societies ensuring that the main contraceptive action of UPA is ovulation impairment without affecting implantation (Rosato et al., 2015). In view of this controversy and on the relative lack of information of the effects of UPA on endometrium, especially under *in vivo* conditions, herein we investigated the effects of a single administration of 30 mg of UPA to women just before FR, when follicular diameter reached ≥20 mm, on the global gene expression profile in endometrial biopsies taken during the window of implantation, seven days after the LH peak (LH+7). Serum pituitary and ovarian hormones were measured and immunohistochemistry (IHQ) studies of key endometrial markers involved in receptivity and implantation were also performed. All results were compared to those of untreated control cycles.

2. Material and methods

2.1. Subjects

The Human Ethical and Scientific Review Committees of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico approved the study, and all participants signed an informed consent form. Healthy women, without pregnancy risk, between 18 and 35 years old, with regular menstrual cycles (28.6 ± 2 days) volunteered to participate in this study. None of them had used hormonal contraceptives or any other medication, including glucocorticoids within 6 months prior to the inclusion in the study. Participants were in good health as determined by medical history, physical examination, and routine screening laboratory analysis.

2.2. Study design

A self-controlled study was carried out in 14 healthy tubal ligated women with normal ovarian function. Each woman served as her own control and was studied longitudinally during two consecutive menstrual cycles. A control-untreated cycle was intended to document the major characteristics of the menstrual cycle and a second was the treated cycle with a single dose of 30 mg UPA. A menstrual cycle was defined as the time elapsed from the first day of a spontaneous menstrual bleeding until the day preceding the next menses. Follicular phase was considered from the first day of bleeding until the day of appearance of LH in urine and the luteal phase from the day of FR until the day before menses began. Menstrual cycle length was defined as normal when it was in the range of 24–35 days and a luteal phase of at least 12 days of length. All participants were admitted to the outpatient clinic of the Institute during the first 10 days of their menstrual cycle. In the treated cycle, each woman received only one dose of 30 mg of UPA (EllaOne, donated by Laboratorios Elea México, S.A. de C.V.) during the preovulatory phase when the size of the leading follicle was ≥20 mm diameter. This time of the cycle was chosen to assure that treatment was done just previous the follicle ruptures when the probability of pregnancy is high. During both study cycles, each woman was instructed to avoid intercourse otherwise a barrier method was recommended, as well as asked to monitor for urinary LH (uLH) every morning starting on the 11th day of the menstrual cycle. Urinary LH was measured using a self-test (Ovu-QUICK™, Quidel, San Diego, CA, USA), with a threshold of 40 mIU/ml. At the time of uLH detection, daily follicle development recordings were performed by transvaginal controlultrasound (TVU) until FR was observed. FR was established as previously described (Batzer, 1986). TVU was performed by the same observer using an Antares Sonoline Siemens ultrasound (Siemens Medical Solutions USA, Pleasanton, CA) equipped with a 7.5 Hz multi-frequency transducer. Women who did not present positive uLH or FR during the control cycle were excluded from the study. Serial blood samples were drawn daily in both study cycles during the periovulatory and the complete luteal phases for measurement of LH, estradiol (E_2) and progesterone (P_4). All serum samples were centrifuged and stored at −20 °C until assayed.

At day LH+7, an endometrial biopsy was taken from all participants during both control and treated cycle. Endometrial tissue was obtained with a Novak curette from the anterior wall of the uterine cavity. Biopsy specimens were prepared for histological or IHQ analysis or frozen in liquid nitrogen to be processed for RNA isolation.

2.3. Hormone assays

Serum concentrations of LH, E_2 and P_4 were measured in duplicate by an immunoradiometric assay for LH (ICN Pharmaceuticals, Inc. CA, USA) and a specific enzyme immunoassay for E_2 and P_4 (DRG Instruments, Germany and Siemens Healthcare Diagnostics, Gwynne, UK, respectively). The intra- and inter-assay coefficients of variation (CV) for all hormone measurements were less than 10%. These CVs were calculated from pools of standard sera at known averages of low and high hormone concentrations.

2.4. RNA isolation and microarray hybridization

Total RNA was extracted from endometrial biopsies according to the technique of Chomczynski and Sacchi (1987) using the TRIzol® reagent (Invitrogen, Carlsbad, CA, USA). The RNA concentration was determined using ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The quality of the RNA was checked on an Agilent BioAnalyzer 2100 (Agilent Technologies, Inc., Palo Alto, CA, USA). Samples with RNA integrity number >9.0 were processed using Affymetrix Whole Transcript Sense Target Labeling Kit (Affymetrix, Santa Clara, USA).

For microarray experiments, target cDNA was prepared according to the Whole-Transcript PLUS (WT) Sense Target Labeling Protocol (Affymetrix, Inc., Santa Clara, CA., USA) as previously described (Lira-Albarran et al., 2017). Briefly: each endometrial RNA sample (200 ng) was reverse transcribed to first-strand cDNA using Superscript II reverse transcriptase primed by a poly(T) oligomer. Second strand cDNA synthesis was followed by an *in vitro* transcription to generate cRNA using T7 RNA polymerase (Affymetrix). The cRNA products were used as templates for a second cycle cDNA synthesis where dUTPs are incorporated to the new strand. The cDNA was fragmented using uracil-DNA glycosidase and apurin apirymidin endonuclease. The fragments (40–70 mers) where then labeled by means of a biotin-labeled deoxynucleotide terminal addition reaction. Labeled single-stranded cDNA was hybridized during 17 h at 45 °C onto GeneChip® Human Gene 2.0 ST Array (Affymetrix, Sunnyvale, CA, USA). This microarray contains 40,716 total RefSeq transcripts for 30,654 NM RefSeq well-stablished annotations. The washing and staining with streptavidin-phycoerithryn were performed in the Affymetrix Fluidics Station 450 and using the Affymetrix Staining Kit (Affymetrix). Finally, the microarrays were scanned for fluorescence signals using a

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