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A single preovulatory administration of ulipristal acetate affects the decidualization process of the human endometrium during the receptive period of the menstrual cycle

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ABSTRACT

In order to get further information on the effects of ulipristal acetate (UPA) upon the process of decidualization of endometrium, a functional analysis of the differentially expressed genes in endometrium (DEG) from UPA treated-*versus* control-cycles of normal ovulatory women was performed. A list of 1183 endometrial DEG, from a previously published study by our group, was submitted to gene ontology, gene enrichment and ingenuity pathway analyses (IPA). This functional analysis showed that decidualization was a biological process over-represented. Gene set enrichment analysis identified *LIF*, *PRL*, *IL15* and *STAT3* among the most down-regulated genes within the JAK STAT canonical pathway. IPA showed that decidualization of uterus was a bio-function predicted as inhibited by UPA. The results demonstrated that this selective progesterone receptor modulator, when administered during the periovulatory phase of the menstrual cycle, may affect the molecular mechanisms leading to endometrial decidualization in response to progesterone during the period of maximum embryo receptivity.

1. Introduction

Recently we described, in ovulatory normal women, the effects of ulipristal acetate (UPA), a selective progesterone receptor modulator, on the global gene expression of endometrial samples taken at the time of maximum receptivity in the menstrual cycle (Lira-Albarran et al., 2017a). Functional analysis of the differentially expressed genes (DEG) showed genomic changes compatible with a non-receptive endometrial phenotype. The human endometrium is subject to cyclic changes occurring during the menstrual cycle under the control of the steroid hormones estradiol and progesterone (Cha et al., 2012; Pawar et al., 2014). Steroid biological responses are mediated via interactions of the steroid with intracellular steroid receptors (Brosens et al., 2004). In the case of progesterone, its receptor (PGR) has two main forms termed PGR-A and PGR-B, both present in the endometrium (Mote et al., 1999). Among the main actions of progesterone on the endometrium is the process of decidualization, which, via the PGR, involves a number of morphological and functional differentiation processes in stromal cells leading to a successful embryo implantation (Bhurke et al., 2016; Ramathal et al., 2010; Wetendorf and DeMayo, 2012).

Interestingly, in the above-mentioned communication from this laboratory, administration of UPA at the time of ovulation induced transcriptional changes of endometrial genes that are involved in implantation. From the list of DEG, by microarray analysis, several canonical pathways were identified, particularly those known to be important in the process of implantation (Choi et al., 2016; Fan et al., 2008; Lee et al., 2013). These data are of importance due to the relative lack and ambiguous information about the effects of UPA on endometrial receptivity (Berger et al., 2015; Mozzanega et al., 2014), in particular under conditions such as emergency contraception. In this study, we wanted to revisit our data and expand our observations on endometrial gene expression in women treated with UPA by looking at genes and proteins involved in decidualization and embryo implantation.

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2. Material and methods

2.1. Subjects and samples

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 between 18 and 35 years old without pregnancy risk and with regular menstrual cycles (28.6 ± 2 days) volunteered to participate in this study. The subjects, including clinical and anthropometric characteristics as well as the study design, were previously described (Lira-Albarran et al., 2017a). Briefly: fourteen tubal ligated normal menstruating women were studied longitudinally during two consecutive menstrual cycles; a control- and a treated-cycle. In the treated-cycle, each woman received one single oral dose of 30 mg of UPA (EllaOne, donated by Laboratorios Elea México, S.A. de C.V.) during the preovulatory phase when the diameter of the leading follicle was ≥20 mm. Daily follicle development recordings were performed by transvaginal ultrasound until follicle rupture (FR) was observed. Women who did not present positive urinary LH or FR during the control or treated-cycle were excluded from the study. At day six after FR, corresponding to seven days after the maximum concentration of LH in serum (LH+7), an endometrial biopsy was taken from all participants during the control- and UPA treated-cycle. Endometrial tissue was obtained with a Novak curette from the anterior wall of the uterine cavity. Biopsy specimens were prepared for histological and immunohistochemistry (IHQ) analysis or frozen in liquid nitrogen for RNA isolation.

2.2. Microarray dataset description

Endometrial data consisted of a selected list of DEG between UPA treated- and control-cycles from a microarray experiment previously published by our laboratory (Lira-Albarran et al., 2017a). Genes in this list were selected according to functional relevance, relative intensity levels and statistical significance.

2.3. Functional analysis

The functional analysis was done by FatiGO of Babelomics 5.0 (http://babelomics.bioinfo.cipf.es) using gene ontology terms (biological processes) generated by the list of DEG (Al-Shahrour et al., 2004). The adjusted p-value was calculated by the exact Fisher test that evaluates the significant overrepresentation of functional terms in the list of DEG in relation to the rest of the human genome after correcting for multiple tests (multiple hypotheses, one for every functional term) using false discovery rate (Colquhoun, 2014). In addition, a gene set enrichment analysis (GSEA: http://www.broadinstitute.org/gsea) was performed using the normalized enrichment score (NES) as primary statistic with a p-value < 0.05 as threshold of statistical significance. Furthermore, a leading edge analysis was done to identify the common genes to one or more canonical pathways (Hung et al., 2012; Subramanian et al., 2005). To understand the effects of UPA on the biofunction decidualization of uterus a network analysis was conducted using the molecule activity predictor tool in Ingenuity Pathway Analysis (IPA: http://www.ingenuity.com) as previously described (Lira-Albarran et al., 2017a,b).

2.4. Quantitative PCR

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). Samples with RNA integrity number > 9.0 were processed using Affymetrix Whole Transcript Sense Target Labeling Kit (Affymetrix, Santa Clara, USA). One μg of high quality total cell RNA from each endometrial sample was reverse transcribed using the Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. Quantitative real time PCR (qPCR) was performed on a Light Cycler® 2.0 Detection System using a TaqMan PCR Master Mix (Applied Biosystems, CA, USA). The oligonucleotides used to amplify every gene were designed with the on-line software of ROCHE (Universal Probe Library Assay Design Center, ROCHE, https://www.roche-applied-science.com), and the sequences verified by BLAST. The sequences of the oligonucleotides used to gene amplification are shown in Table S1. The comparative Ct method (ΔΔCt) was used to quantify expression of target genes (Schefe et al., 2006) and normalized to *ACTB* used as the housekeeping gene (Kozera and Rapacz, 2013).

2.5. Endometrial morphological analysis

All evaluations were performed under blinded conditions. Endometrial differentiation was assessed by histological dating using the criteria described by Noyes et al. (1975). The readings of specimens were considered abnormal if glandular maturation was delayed by three or more days from the chronological day of cycle. The chronological day of cycle was determined as the number of days from ultrasound documented FR to biopsy.

For IHQ, paraformaldehyde-fixed, paraffin-embedded endometrium were sectioned at a thickness of 4 μm, deparaffinized and stripped of endogenous peroxidase. Antigen retrieval was done by placing the slides in citrate buffer at a pH of 6.0 (ImmunoDNA Retriever Citrate, BioSB, Inc. Santa Barbara CA, USA.) and heated in a pressure cooker for 5 min. Sections were incubated overnight at 4 °C in the presence of primary antibodies at a final dilution depending on the antigen (Table S2). After washing, sections were incubated with peroxidase-labeled secondary antibodies and stained with the 3, 3′diaminobenzidine peroxidase substrate kit (Vector Laboratories, Burlingame, CA, USA) and lightly counterstained with hematoxylin. Slides were subjected to qualitative and quantitative assessment of both, glands and stroma.

Digital pictures were obtained on an Olympus BX51 (Olympus Co. Model BX51RF, Tokyo, Japan). Ten pictures from stromal and 15 for endometrial glands were taken for each slide. Images were analyzed using a digital image analyzing software (ImageJ, U.S. National Institute of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/) and color deconvolution Plug-in as previously described (Ruifrok and Johnston, 2001). Images were converted into black-and-white and black percentages were evaluated. Incubations in the absence of the primary antibody were used as negative controls.

2.6. Endometrial cell culture

The cells used in this study are primary human stromal endometrial cells donated by Dr. Robert Taylor (Department of Obstetrics and Gynecology, Wake Forest School of Medicine Winston Salem, NC, USA) from biopsies of normal endometrium (Yu et al., 2016). The cell lineage was confirmed, in most, if not all the cells, as stromal cells by immunocytochemistry assessing the expression of Vimentin by the use of a specific antibody (Fig. S1), as previously described (Duval et al., 2017; Yu et al., 2015). These cells were cultured in DMEM/F-12 (Invitrogen Life Technologies, Inc., Carlsbad, CA) medium supplemented with charcoal-stripped 10% fetal calf serum (Hyclone, Gent, Belgium), 2 mM L-glutamine, 2 mM Na-pyruvate, 100 U/L penicillin and 100 μg/L streptomycin. At confluence, cells were cultured in the presence or absence of 10^{-7} M medroxyprogesterone acetate (MPA) in medium containing UPA or mifepristone (MFP) at the dose of 10^{-6} M during 10 days. All incubations including the vehicle contained 8-Br-cAMP (5×10^{-4} M). At the end of the incubation period, total RNA was extracted and four markers of endometrial decidualization (*PRL, IGFBP1, PGR* and *HAND2*) were analyzed by RT-qPCR as described above.