Contraception

Contraception 93 (2016) 65–69

Original research article

Effect of oral contraceptives and doxycycline on endometrial MMP-2 and MMP-9 activity

Bliss Kaneshiro[a,*], Alison Edelman[b], Chandravanu Dash[c], Jui Pandhare[c], Faapisa M. Soli[d], Jeffrey T. Jensen[b]

[a]John A. Burns School of Medicine, University of Hawaii at Manoa, 1319 Punahou Street, Suite 824, Honolulu, HI 96826, USA
[b]Oregon Health & Science University, 3181 SW Sam Jackson Park Road, UHN 50, Portland, OR 97239, USA
[c]Meharry Medical College, 1005 Dr. D. B. Todd Jr. Boulevard, Suite 5131, Nashville, TN 37208, USA
[d]RMATRIX, John A. Burns School of Medicine, University of Hawaii at Manoa, 651 Ilalo Street, Medical Education Building, Office of the Dean, 2nd Floor, Honolulu, HI 96813, USA

Received 13 March 2015; revised 1 September 2015; accepted 17 September 2015

Abstract

Objectives: To describe the effect of combined oral contraceptives (COCs) on matrix metalloproteinases MMP-2 and MMP-9 activity and compare MMP activity in women taking a COC with or without doxycycline.
Study design: Subjects (n=20) underwent endometrial biopsies (1) in the late luteal phase of a baseline cycle prior to initiating COCs, (2) on days 19–21 while taking COCs in a standard 28-day cycle (7-day hormone-free interval) and (3) on days 26–28 while taking active COCs continuously for a 28-day cycle. During the continuous COC cycle, they were randomized to receive daily subantimicrobial dose doxycycline 40 mg or placebo.
Results: Compared to baseline, COC treatment increased MMP-2 (p<.001) and MMP-9 (p<.001). MMP activity was lower in subjects taking a COC with doxycycline compared to those receiving placebo although only significantly lower for MMP-2 latent form (p=.002).
Conclusions: Unscheduled bleeding with COCs may be the result of increased endometrial MMPs. Sample size limitations prevent us from determining how doxycycline affects MMP activity in COC users.

Keywords: Combined oral contraceptives; Matrix metalloproteinase-2 (MMP-2); Matrix metalloproteinase-9 (MMP-9); Doxycycline; Endometrial biopsy

1. Introduction

Unscheduled bleeding is a common side effect of cyclic, extended and continuous combined oral contraceptives (COCs) [1]. Recent investigations aimed at decreasing unscheduled bleeding with hormonal contraceptives have focused on preventing endometrial degradation on a molecular level by altering matrix metalloproteinases (MMPs) [2–5]. MMPs are present throughout the endometrium with higher MMP activity associated with greater endometrial degradation and subsequent bleeding [6,7].

Understanding the molecular effects of COCs and MMP inhibitors like doxycycline on the endometrium may contribute to our understanding of why women experience unscheduled bleeding and what can be done to decrease this unwanted side effect. In a randomized placebo-controlled trial, we found that doxycycline did not decrease bleeding when it was administered as a treatment measure once bleeding had started [8]. We hypothesized that once endometrial degradation began, inhibition of MMP activity with doxycycline was not sufficient to stop bleeding, but if MMP activity could be inhibited prior to the onset of endometrial degradation, this could result in less unscheduled bleeding. In a follow-up randomized placebo-controlled trial, we demonstrated that prophylactic administration of subantimicrobial doxycycline (40 mg daily) allowed women taking a COC in a continuous manner to achieve menstrual suppression sooner than those who took a placebo [9].

Abbreviations: COC, combined oral contraceptive; MMP, matrix metalloproteinase

* Corresponding author. Tel.: +1-808-203-6500.
E-mail address: blissk@hawaii.edu (B. Kaneshiro).

http://dx.doi.org/10.1016/j.contraception.2015.09.006
0010-7824/

Despite containing both an estrogen and a progestin, all COCs have a progestin-dominant effect on the endometrium. Exogenous progestin, like those found in COCs, may up-regulate MMP activity [4]. Other medications like the tetracyclines inhibit MMPs. Independent of its antimicrobial properties, doxycycline, at low, subantimicrobial doses, is a potent MMP inhibitor [10,11].

We elected to study MMP-2 and MMP-9 based on their known roles in endometrial degradation and menstruation and their hypothesized role in unscheduled bleeding associated with hormonal contraceptive use. MMPs are grouped according to their dominant structure into collagenases, gelatinases, stromelysins and membrane-bound types and correspondingly degrade those extracellular matrix components [12]. MMP-9 and MMP-2 are gelatinases that degrade collagens IV and V, elastin and gelatin components of the extracellular matrix, as well as the subendothelial basement membrane thereby increasing vessel fragility. The hypothesized outcome is increased endometrial bleeding [13].

The purpose of the current study was to describe MMP-2 and MMP-9 activity in endometrial biopsies collected (1) prior to initiating COCs, (2) while taking COCs in a standard 28-day cycle (7-day hormone-free interval), (3) while taking 28 days of hormonally active COCs and (4) while taking 28 days of hormonally active COCs with doxycycline. We hypothesized the progestin-dominant effect of COCs would result in increased in MMP activity from baseline. Based on doxycycline's actions as an MMP inhibitor, we also hypothesized that MMP activity would be lower in the group taking a COC with doxycycline compared to those taking a COC alone.

2. Materials and methods

2.1. Subjects and sample collection

A prospective randomized study (Committee on Human Subjects #19375, Clinicaltrials.gov NCT01469585) was conducted in Honolulu, Hawaii, between December 2011 and May 2012. Ovulatory women (progesterone level≥3.0 ng/mL, days 18–20), 18–45 years of age with no contraindications to COCs or doxycycline, were invited to participate. Exclusion criteria included use of an intrauterine device (IUD) or contraceptive implant (within 4 weeks) or depot medroxyprogesterone acetate (within 9 months), smoking or irregular menstrual cycles.

Each participant had three endometrial biopsies over three 28-day cycles (84 days total). A baseline biopsy was done prior to initiating any medications on days 19–21 of the menstrual cycle (baseline cycle). On the first day of spontaneous menses of treatment cycle 1, participants started a daily COC (20 mcg ethinyl estradiol/100 mcg levonorgestrel; Lutera®, Watson Pharma, Inc., Corona, CA). The second biopsy was performed on days 19–21 (treatment cycle 1). On day 21 of treatment cycle 1, participants began the 7-day placebo week as they would with typical COCs.

During treatment cycle 2, participants took hormonally active pills for all 28 days and were randomized to either daily controlled-release subantimicrobial dose of doxycycline 40 mg (Oracea®, Galderma Laboratories L.P., Fort Worth, TX) or no additional medication using simple randomization. The third endometrial biopsy was performed on days 26–28 (treatment cycle 2). The snap frozen samples were stored in a −80°C freezer and shipped to Meharry Medical College (Nashville, TN) for analysis.

2.2. Extraction and activity assay of MMPs

Samples were homogenized on ice in buffer containing 50 mM Tris–HCl, 500 mM NaCl, 5 mM $CaCl_2$ and 1% Triton X-100, pH 7.6. The homogenates were centrifuged at 11,000*g* (30 min, 4°C) and the supernatants were assayed for MMP activity using gelatin zymography. An aliquot of the supernatant was assayed for protein concentration using the BCA kit (Pierce, USA). Gelatinase zymography was performed using 10% Novex precast polyacrylamide gel (Invitrogen) in the presence of 0.1% gelatin. Equal amounts of total protein were loaded per well and gels were electrophoresed under nonreducing conditions. In addition, a sample containing 2.5 ng each of MMP-2 and MMP-9 (Enzo Life Sciences, USA) as positive control was run together with experimental samples on each gel. After electrophoresis, gels were treated with renaturing buffer (Invitrogen), followed by incubation in developing buffer (Invitrogen) at 37°C. Gels were stained with SimplyBlue SafeStain (Invitrogen) and washed. Densitometry analyses were carried out using a Bio-Rad gel documentation system with Image Lab Software.

2.3. Statistical analysis

To describe differences in MMP-2 and MMP-9 activity between subject taking a COC along with doxycycline (doxycycline group, biopsy 3) and those taking a COC alone (control group biopsy 3), a *t* test was used. To describe the effects of COCs on MMP activity, we compared MMP activity between the baseline biopsy (biopsy 1) and the biopsy while taking COCs (treatment cycle 1, biopsy 2) using a paired *t* test. All analyses were performed with Statistical Package for the Social Sciences version 16.0 (Chicago, IL). A sample size of 20 was selected to give reasonable estimates of relative MMP activity.

3. Results

Of the 37 subjects screened, 20 were randomized (Fig. 1). Most women were Asian (30%) or Caucasian (35%), nulliparous (85%) and normal weight (mean body mass index: 23.9 kg/m^2). No significant differences in demographic characteristics emerged between the groups. MMP activity increased significantly when subjects started a COC (baseline biopsy 1 versus treatment cycle 1, biopsy 2) for