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The effect of 7 days of letrozole pretreatment combined with misoprostol on the expression of progesterone receptor and apoptotic factors of placental and decidual tissues from first-trimester abortion: a randomized controlled trial☆

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Abstract

Objective: To evaluate if letrozole-induced suppression of estradiol reduces progesterone receptor expression and apoptosis in the first-trimester placenta.

Study Design: We performed a double-blinded, randomized, placebo-controlled trial. We randomized 20 women requesting first-trimester abortion with gestation up to 63 days to receive either letrozole 10 mg daily or placebo pretreatment for 7 days before administrating 400 mcg of vaginal misoprostol followed by suction abortion. We collected the placental and decidual tissues on which we performed immunohistochemical staining for progesterone receptor and apoptotic markers (active caspase 3, caspase 3, Bcl2, CD95, fas ligand) and determined H-scores of each based on the intensities of staining. We performed terminal deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick end labeling (TUNEL) assay for apoptosis in the samples of four women to confirm the findings from apoptotic markers.

Results: We excluded one woman in the letrozole group from the analysis because she had passage of abortus after taking letrozole, leaving 19 women (9 in the letrozole group, 10 in the placebo group) for analysis. There was no significant difference in the H-scorings of progesterone receptor and apoptotic markers, as well as proportion of apoptotic cells on TUNEL assay between the two groups. The H-scores for the progesterone receptor were 8.17±2.67 (mean±SD) in the letrozole group and 9.01±2.82 in the placebo group ($p=0.36$).

Conclusion: We did not detect a difference in the expression of progesterone receptor and apoptotic markers in placental and decidual tissues after letrozole pretreatment for 7 days in first-trimester abortion.

Implications: We did not confirm the hypothesis that letrozole reduces progesterone receptor expression and induces apoptosis in the first-trimester placenta. Further studies are required to allow better understanding of the mechanism by which estrogen suppression following the use of letrozole can lead to improved abortion rate in the first trimester.

Keywords: Letrozole; Misoprostol; Medical abortion; Progesterone receptor; Apoptosis

1. Introduction

Mifepristone combined with misoprostol is the preferred regimen for medical abortion in the first trimester with a complete abortion rate through 63 days gestation of 95–98% [1–8]. However, mifepristone is expensive and is not available in many countries, limiting the use of this regimen. The complete abortion rate of misoprostol alone regimen is lower. It generally ranges from 60 to 95%, depending on the regimen, definition and time to measurement of success and

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duration of pregnancy [8–15]. One randomized trial showed that combination of mifepristone and misoprostol was significantly more effective for abortions ≤ 56 days than misoprostol alone (success rate 95.7% vs. 88.0%, $p < 0.05$) [8].

Letrozole, a third-generation selective aromatase inhibitor, has been investigated as an adjunct to misoprostol for first trimester medical abortion. Our group has demonstrated in a randomized controlled trial that combined use of letrozole 10 mg daily for 3 days followed by 800 mcg of vaginal misoprostol resulted in increased complete abortion rate when compared to misoprostol. The abortion rate of letrozole and misoprostol in pregnancies up to 63 days gestation was up to 86.9% (93.3% in pregnancies up to 49 days gestation) [16]. We performed another pilot study using 7 days letrozole pretreatment with up to 95% abortion rate [17].

The mechanism by which letrozole pretreatment improves the abortion rate in medical abortion is unclear. Our group has demonstrated that letrozole suppresses the expression of estrogen receptor-α and progesterone receptor (PR) transcripts in the placenta in the second trimester [18], but information on the first-trimester placenta is lacking. We postulate that letrozole-induced suppression of estradiol reduces PR expression in the first-trimester placenta, leading to reduction of their response to progesterone, an effect similar to but the mechanism different to that of mifepristone, which directly binds to the PR to block its activity. The reduced response to progesterone may then induce apoptosis in the placenta and increases the success rate of medical abortion. We performed the present study to address this hypothesis.

2. Material and methods

2.1. Study population

We performed a double-blinded, randomized, placebo controlled trial to study the effect of letrozole compared to placebo in early pregnancy on the trophoblasts and decidual tissues including the expression of PR and apoptotic markers.

The recruitment took place in April and May 2013. We recruited 20 women attending the General Gynaecology Clinic of the Department of Obstetrics and Gynaecology, the University of Hong Kong, requesting legal abortion at gestational age up to 63 days. Inclusion criteria included history of good health, age of women at 18 years or above and an intrauterine pregnancy with duration of pregnancy not more than 63 days as confirmed by transvaginal ultrasound on the day of study drug administration. We excluded women who had multiple pregnancies, uterine fibroids, presence of intrauterine device, diastolic pressure over 95 mmHg, history or evidence of adrenal pathology, steroid-dependent cancer, porphyria, bronchial asthma, arterial hypotension, thromboembolism, severe or recurrent liver disease or pruritus of pregnancy, regular use of prescription drug prior to the study, known allergy to letrozole or misoprostol and those who were breastfeeding.

Subjects who fulfilled the selection criteria and were willing to participate gave their informed written consent. The Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster approved the study. The trial was registered on the ClinicalTrials.gov, with Identifier NCT02457312.

2.2. Randomization and treatment regimen

After detailed history, physical examination and transvaginal ultrasound examination to confirm gestational viability and gestational age, we recruited eligible women. We randomized the recruited women to receive either letrozole (Farmoz, Portugal) 10 mg (letrozole group) or placebo tablets (placebo group) daily for 7 days before surgical abortion. A research nurse not involved in the clinical care of patients prepared and numbered the packages of letrozole and placebo tablets according to a computer-generated randomization schedule in 1:1 ratio with a block size of 4. The placebo tablets looked identical to the letrozole tablets, and their packages also looked identical. Until the completion of the study, both the patients and clinicians were blinded to the group assigned.

We instructed the women to take the study drug (placebo or letrozole 10 mg) once daily at the same time in the morning for 7 days. We took blood for serum estradiol, progesterone and human chorionic gonadotropin (hCG) levels before administration of letrozole or placebo and again at around 8 a.m. on the day of suction evacuation. After taking 7 days of letrozole or placebo, the women underwent surgical abortion on day 8. We gave 400 mcg of vaginal misoprostol for cervical priming before the procedure. We collected the suction aspirates, which contained both placental and decidual tissues, for immunohistochemical (IHC) staining and in-situ terminal deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick end labeling (TUNEL) assay.

2.3. IHC staining

We fixed the tissues obtained (placenta and decidua) in 4% paraformaldehyde for paraffin embedding and then cut them into sections for IHC analysis.

We performed IHC staining for PR and apoptotic markers as described previously [18]. In short, we incubated primary anti-PR (1:100 dilution, Dako), anti-CD95 (1:300 dilution, abcam), anti-fas ligand (1:400 dilution, abcam), anti-caspase-3 (1:400, abcam), anti-active caspase 3 (1:200, abcam) and anti-Bcl-2 (1:200, abcam) antibodies in 10% donkey serum with the tissue sections at 4°C overnight. We then thoroughly washed the tissue sections with phosphate-buffered saline and then incubated them with a secondary antibody in 10% donkey serum, followed by StrepABComplex. A positive brown signal was visualized with 3,3-diaminobenzidine (Dako) as the substrate. We then counterstained the sections in light haemotoxylin, dehydrated, cleared and mounted them.

2.4. In-situ TUNEL assay

We randomly sampled four women (two in each group) to confirm the findings of apoptotic markers immunostaining by the *in-situ* TUNEL assay. We used the ApopTag® Plus Fluorescein *In Situ* Apoptosis Detection Kit (Chemicon