Life Sciences 96 (2014) 46–52

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Cilostazol administered to female mice induces ovulation of immature oocytes: A contraceptive animal model

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ARTICLE INFO

Article history:
Received 5 October 2013
Accepted 13 December 2013

Keywords:
Contraceptives
Ovulation
Immature oocytes
Superovulation
Fertility
Cilostazol

ABSTRACT

Aims: Both Cilostamide and Org 9935 are phosphodiesterase 3A (PDE3A) inhibitors that were evaluated in rodents and monkeys for their non-steroidal contraceptive properties. Although both compounds inhibited oocyte maturation, an adverse effect on heart rate was observed. Cilostazol (CLZ, Pletal®) is a safe PDE3A inhibitor that was recently reported to block pregnancy in naturally cycling mice. In this study, the dose, frequency, time of administration, and reversibility effects of CLZ on oocyte maturation were defined using superovulated mice.
Main methods: Superovulated mice were gavaged once or twice with 0, 7.5, or 15 mg CLZ at various times around the ovulatory stimulus. Ovulated oocytes were then evaluated for maturational stages.
Key findings: CLZ resulted in mice ovulating significant numbers of immature oocytes when administered anytime between 9 h before the ovulatory stimulus and 2 h after the stimulus. This inhibitory effect was greater when CLZ dose was increased, administered twice or closer to the time of the ovulatory stimulus. Conversely, ovulated immature oocytes resumed maturation in oviducts when CLZ dose was reduced, administered once and away from the time of the stimulus.
Significance: Controlling CLZ dose, frequency, and time of administration produced mice ovulating immature oocytes at different stages, which may be an advantage over the conventional collection of immature oocytes from ovaries. More importantly, the capability of a clinically approved PDE3A inhibitor, with a reasonable margin of safety, to arrest oocyte maturation over a wide range of administration times and at doses extrapolated from human therapeutic doses demonstrates the contraceptive capacity of CLZ.

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Introduction

In the late 19th century, several investigators had linked the development of the corpus luteum to the lack of ovulation in different species. In 1916, Herrmann and Stein reported that lipid extracts from corpora lutea inhibited ovulation in rats. During the 1920s, an Austrian professor of physiology named Ludwig Haberlandt put these observations into the context of contraception and formulated the present concept of a steroidal contraceptive pill (Goldzieher and Rudel, 1974; Haberlandt, 2009). The current steroidal contraceptives contain synthetic estrogen and progesterone that act centrally and peripherally to produce contraception. Centrally, they use the hormonal negative feedback effects of progesterone and estrogen to inhibit the synthesis and release of luteinizing hormone and follicle-stimulating hormone from the anterior pituitary, which impairs follicular development and ovulation. Peripherally, the steroidal contraceptives alter the female reproductive tract in a manner that does not support oocyte fertilization and embryonic implantation (Bronson, 1981). Although no birth control method is 100% effective (except for abstinence), steroidal contraceptives have repeatedly been shown to control population growth. However, this method of steroidal contraception interferes with ovarian steroidogenesis and is associated with hypertension (Atthobari et al., 2007), myocardial infarction (Tanis et al., 2001), venous thromboembolism (Blanco-Molina et al., 2012), and breast cancer (Iatrakis et al., 2011; White et al., 1994). While scientific research has developed many antibiotics and antihypertensive drugs using different pharmacological approaches, the pharmacological development of contraceptives that are not steroidal in nature has yet to be achieved. Such non-steroidal contraceptives would be free of side effects that are steroidal in nature. It would also aid those women that cannot tolerate steroidal contraceptives, especially those with a history of cancer and cardiovascular diseases.

An inhibitory role for cAMP on oocyte spontaneous maturation in vitro was suggested in 1974 by Cho and coworkers (Cho et al., 1974). Numerous studies have then shown that inhibition of phosphodiesterase 3A (PDE3A) in oocytes of many species, including humans, is capable of maintaining high intraoocyte cAMP concentrations and consequently arresting oocyte maturation in vitro. PDE3A inhibitors were also evaluated in vivo. Although PDE3A inhibitors were capable

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0024-3205/$ – see front matter. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.lfs.2013.12.022

of inhibiting oocyte maturation in rodents (Wiersma et al., 1998) and primates (Jensen et al., 2005, 2010) without influencing ovulation and ovarian steroidogenesis, they increased heart rates in both species because oocytes and myocytes both express PDE3A.

Cilostazol (CLZ) is a PDE3A inhibitor that is prescribed for patients with intermittent claudication disease in the USA and Europe. The compound is also prescribed as an antiplatelet/antithrombotic agent in Japan and other Asian countries. Although intermittent claudication and platelet aggregation are common in people with relatively advanced age, administration of CLZ (Pletal®) in seniors is common and safe (Liu et al., 2001). CLZ has recently been reported to block pregnancy in naturally cycling mice (Taiyeb et al., 2013). The aim of the present study was to investigate the effect of dose, frequency, and time of CLZ administration on oocyte maturation using the superovulated mouse model. The reversible inhibitory effect of CLZ on oocyte maturation in vivo was also studied.

Materials and methods

Animals and reagents

Swiss Webster mice (8–10 weeks old) were purchased from Harlan Laboratories (Houston, Texas). Mice were housed in groups of 5–8 mice per cage under controlled temperature (23 °C) and light/dark cycle (14/10 h). Food and water were provided ad libitum. All experiments and procedures were approved by the Texas A&M University Institutional Animal Care and Use Committee.

All mice were injected intraperitoneally (ip) with 7.5 IU pregnant mare serum gonadotropin (Folligon®) and 47 h later with 7.5 IU human chorionic gonadotropin (hCG, Chorulon®). Both Folligon® and Chorulon® were purchased from Intervet Inc. (Summit, New Jersey). CLZ was purchased from LKT Laboratories (St. Paul, Minnesota) whereas Leibovitz's L-15 medium was purchased from Invitrogen (Grand Island, New York). All other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Cilostazol dose determination

The doses of CLZ evaluated in this study were extrapolated from the pharmacokinetics of CLZ in humans. CLZ was found capable of inhibiting mouse oocyte maturation in vitro at concentrations of 390.5, 781, and 1665 μg/L (Taiyeb et al., in press). These in vitro inhibitory concentrations of CLZ were similar to plasma concentrations of CLZ reported in people who are taking therapeutic doses of CLZ (Bramer et al., 1999). Starting from these vitro inhibitory concentrations, as the desired initial mouse plasma concentrations and the central volume of distribution of 20.5 L reported by Yoo et al. (2010) in humans, the 7.5 and 15 mg doses of CLZ tested in this study were determined.

Experimental design

The first experiment was designed to investigate the effect of time of administration of a low dose of CLZ (7.5 mg) with respect to time of administration of hCG on oocyte maturation in superovulated mice. Superovulated mice were treated with 7.5 mg CLZ, dissolved in 0.1 ml DMSO, at 9 h pre-hCG, 7 h pre-hCG, 4 h pre-hCG, at the same time as hCG, 2 h post-hCG, or 4 h post-hCG. Control animals received 0.1 ml DMSO at the same times as their corresponding groups treated with CLZ. Ovulated oocytes from control and treated mice were then collected 13–13.5 h post-hCG (early collection) and scored for meiotic maturation using a stereomicroscope (Nikon, SMZ 1500 model) or an inverted microscope (Olympus, IX71 model).

The second experiment was designed to investigate the effect of time of administration of a high dose of CLZ (15 mg) with respect to time of administration of hCG on oocyte maturation in superovulated mice. Mice were treated with 15 mg CLZ, dissolved in 0.15 ml DMSO, at 9 h pre-hCG, 7 h pre-hCG, 4 h pre-hCG, at the same time as hCG, 2 h post-hCG, or 4 h post-hCG. Control animals received 0.15 ml DMSO at the same times as their corresponding groups treated with CLZ. Ovulated oocytes from control and treated mice were collected as described in the 1st experiment.

The third experiment was designed to investigate the effect of time of administration of multiple doses of CLZ with respect to time of administration of hCG on oocyte maturation in superovulated mice. Mice were treated with 7.5 mg CLZ dissolved in 0.1 ml DMSO at 4 h pre-hCG and 2 h post-hCG. Another group of mice was treated with 7.5 mg CLZ dissolved in 0.1 ml DMSO at the same time as hCG and 6 h post-hCG. Control animals received 0.1 ml DMSO at the same times as their corresponding groups treated with CLZ. Ovulated oocytes from control and treated mice were collected as indicated in the 1st experiment.

The fourth experiment was designed to investigate the reversible inhibitory effect of CLZ on oocyte maturation in superovulated mice. Mice were treated with 7.5 mg CLZ 9 h pre-hCG, 7 h pre-hCG, or 4 h pre-hCG. Ovulated oocytes were then collected 14.5-15.5 h post-hCG (late collection) instead of the early collections. Similarly, late collections for ovulated oocytes were also conducted in mice treated with 15 mg CLZ dose at 4 h pre-hCG. The effect of late collections of ovulated oocytes was also investigated in mice treated with 7.5 mg CLZ 4 h pre-hCG and 2 h post-hCG or at the same time as hCG and 6 h post-hCG.

Oocyte retrieval

Anesthetized animals were sacrificed by cervical dislocation and oocytes were collected 13–13.5 h (early collection) or 14.5–15.5 h (late collection) post-hCG injection. Ovaries and oviducts were excised and placed into 2 ml of Leibovitz's L-15 medium supplemented with 10% bovine serum albumin and 4.2 μM CLZ to prevent spontaneous oocyte maturation. The visibly distended region of the oviductal ampulla was punctured, and the ovulated cumulus enclosed oocyte complexes were collected and denuded in 1 ml of 0.6% bovine hyaluronidase enzyme for less than 1.5 min with occasional pipetting. Denuded oocytes were then scored for maturational status as germinal vesicle (GV), metaphase I (MI), or metaphase II (MII) as previously described (Donahue, 1968). A Nikon SMZ1500 stereomicroscope and Olympus IX71 inverted microscope were used to evaluate oocyte maturational status.

Statistics

The two-way ANOVA test was used to evaluate the effect of interaction between the two main effects of time and dose of administration of CLZ on oocyte maturation. When the two-way ANOVA test indicated a significant interaction between the two main effects, the Bonferroni post hoc test was used to evaluate the statistical differences among the simple main effects. If an interaction was not detected, the independent-sample *t*-test and the one-way ANOVA test followed by the Bonferroni post hoc test were used to evaluate the statistical differences among mean numbers of ovulated oocytes resulting from different treatments and times of administration, respectively. The independent-samples *t*-test was also used to evaluate statistical differences between mean numbers of ovulated GV, MI, or MII oocytes resulting from early or late collections of oocytes from mice treated with 7.5 or 15 mg CLZ. Eight mice were used for each treatment group, and all data are presented as mean ± SEM percent. The threshold for determining a significant difference was set at $P < 0.05$, and SPSS 14.0 software (SPSS Inc., Chicago, IL) was used to carry out the statistical analyses.

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