Postcoital treatment with levonorgestrel does not disrupt postfertilization events in the rat

A.L. Müller, C.M. Llados, H.B. Croxatto*

Pontificia Universidad Católica de Chile, Facultad de Ciencias Biológicas, Unidad de Reproducción y Desarrollo, Av. Alameda Bernardo O'Higgins 340, Santiago, Chile

Received 28 October 2002; received in revised form 8 January 2003; accepted 13 January 2003

Abstract

Levonorgestrel (LNG), a progestin widely used for regular hormonal contraception, is also used for emergency contraception (EC) to prevent pregnancy after unprotected intercourse. However, its mode of action in EC is only partially understood. One unresolved question is whether or not EC prevents pregnancy by interfering with postfertilization events. Here, we report the effects of acute treatment with LNG upon ovulation, fertilization and implantation in the rat. LNG inhibited ovulation totally or partially, depending on the timing of treatment and/or total dose administered, whereas it had no effect on fertilization or implantation when it was administered shortly before or after mating, or before implantation. It is concluded that acute postcoital administration of LNG at doses several-fold higher than those used for EC in women, which are able to inhibit ovulation, had no postfertilization effect that impairs fertility in the rat. © 2003 Elsevier Inc. All rights reserved.

Keywords: Levonorgestrel; Emergency contraception; Ovulation; Fertilization; Implantation; Rat

1. Introduction

Levonorgestrel (LNG), a progestin widely used for regular hormonal contraception, is also used for emergency contraception (EC), alone or combined with estrogen. Hormonal EC is taken after unprotected intercourse in order to prevent pregnancy. The mode of action of EC has become the subject of heated debate in several Latin American and Caribbean countries. The main question is centered on whether or not EC prevents pregnancy by interfering with postfertilization events. This issue is of importance for many people, considering that a new human life begins at the time fertilization is completed. Accordingly, interference with postfertilization events would lead to loss of human life.

When a woman uses EC she does not know whether she takes the pins before or after ovulation and before or after fertilization. For ethical and logistical reasons, it has not been possible to segregate groups of women who take EC after fertilization in order to assess its effect on the establishment of pregnancy. Hence, there is no direct evidence, in favor or against, that acute treatment with LNG prevents pregnancy by interference with postfertilization events.

On the other hand, animal experimentation allows segregating groups treated before or after critical events, such as ovulation, fertilization and implantation in order to define the contribution of pre- and postfertilization events to the contraceptive efficacy of the drug. Even though extrapolation of the results to the human has considerable limitations, experiments in animals often shed light on possible mechanisms operating in the human.

It is known that acute treatment with LNG administered during the follicular phase can inhibit or delay ovulation in various species, including the human [1]. Also, acute treatment with LNG administered within the first 10 h after intercourse decreased the number of spermatozoa recovered from the uterine cavity in women [2]. To our knowledge, no such effect has been described in animals. Furthermore, its action when given after mating is not well documented. Therefore, we undertook experiments in animals to investigate the possible occurrence of postfertilization effects.

In this paper, we describe the effects of acute treatment with LNG given either before or after ovulation or fertilization, or before implantation upon fertility parameters in the rat. The main intervention variable was the time of administration relative to ovulation, mating and fertilization.
The measured variables were percentage of animals ovulating, mean number of ovulated eggs, mean percentage of fertilized eggs and mean number of implanted embryos.

2. Material and methods

2.1. Animals

Adult Sprague Dawley female rats weighing approximately 200 g and adult male rats 5-7 months old were used. All animals were kept in the same room with water and pet chow ad libitum, illumination from 7:30 a.m. to 9:30 p.m. and temperature 20-24ºC.

Females were submitted daily to vaginal smears. Estrous cycle stages were classified according to Turner J3 J. In order to obtain pregnant animals, each proestrus or estrous female was caged individually with two intact males for 1 h or all night. At the end of this period, the presence of semen, blood or a plug in the vagina and/or the presence of spermatozoa in the vaginal smear verified mating. If mating occurred, the day of estrus was designated day 1 of pregnancy (P1).

Animal care and experimental procedures were done according with the ethical guidelines of the Institutional Ethics Committee.

2.2. LNG solutions

Crystalline LNG was obtained from Norplant implants (Leiras, Turku, Finland) or from Schering AG, Berlin, Germany. LNG was dissolved in 100 ul ethanol (Merck, Darmstadt, Germany) and was further diluted with 1,2-propanediol (Sigma, St. Louis, MO, USA) to a final concentration of 100 ug/mL.

2.3. Treatments

Each rat was injected subcutaneously with LNG 50 ug/kg body weight per injection or with vehicle, except in one control group that was left undisturbed. This dose is approximately four times higher than the dose used for EC and its bioavailability is presumed to be higher due to systemic administration. This dose was given one to four times with 12-h intervals. The timing of treatment relative to ovulation, mating, fertilization and implantation is summarized in Figs. 1 and 2.

2.4. Effect of preovulatory treatment upon ovulation

Each rat was injected with LNG or vehicle twice per day (8:00 a.m. and 8:00 p.m.) at both diestrus and proestrus [Group 1 (G1)] or with a single injection at 8:00 p.m. of diestrus (G2) or at 8:00 a.m. of proestrus (G3). These rats were killed at estrus in order to assess percentage of animals ovulating and mean number of ovulated eggs per ovulating rat.

2.5. Effect of treatment before mating and ovulation upon fertilization and implantation

The results of the previous experiment indicated that a single injection of LNG in proestrus was unlikely to affect ovulation. Therefore, it was feasible to study effects of preovulatory treatment upon fertilization and implantation.

Proestrus rats were injected with LNG or vehicle once at 4:00 p.m. Each rat was caged with two males from 9:30 to 10:30 p.m. If mating was verified, the female was isolated and killed on P2 in order to assess the percentage of fertilized eggs (G4). If mating had not taken place by 10:30 p.m., the female remained overnight with the males. When mating was verified the following morning, the female was isolated and killed on P2 in order to assess the number of implanted embryos (G5).
Failure to mate was infrequent and females that failed to mate were discarded.

2.6. Effect of treatment after mating and before ovulation upon fertilization and implantation

Proestrous rats were caged with males from 9:00 to 10:00 p.m. Those that mated were injected with LNG at 11:00 p.m. (G6) or with LNG or vehicle at 11:00 p.m. and again 12 h later (G7). These rats were killed on P2 or P12.

2.7. Effect of treatment after ovulation and before mating upon fertilization and implantation

Females were injected with LNG or vehicle once at 8:00 a.m. on the day of estrus and immediately caged with males until 9:00 a.m. (G8). Those that mated were isolated and killed on P2 or P12.

2.8. Effect of treatment after ovulation and mating upon fertilization and implantation

Females were caged with males from 8:00 to 9:00 a.m. on the day of estrus. If mating was verified, the female was injected immediately with LNG or vehicle and killed on P2 or P12 (G9).

2.9. Effect of treatment after fertilization upon implantation

Proestrous rats were caged with males overnight. Mated females were injected twice with LNG or vehicle at 11:00 p.m. of P1 and at 11:00 a.m. of P2 (G10) or at 11:00 p.m. of P3 and at 11:00 a.m. of P4 (G11) and killed on P12.

A separate assessment (not shown) determined that after mating occurring overnight, fertilization was completed by midmorning in our rat colony. Thus, both of these treatments were given after fertilization had taken place.

2.10. Assessment of ovulation, fertilization and implantation

Unmated rats were killed by an overdose of ether at noon of estrus to assess the number of oocytes present in their oviducts. Mated rats were killed between 3:00 and 4:00 p.m. on P2 or P12 to assess the number of fertilized and unfertilized eggs and the number of implanted embryos, respectively. At estrus or P2, oviducts were removed and flushed with saline to count the number of eggs using a low magnification (250X). When necessary, cumulus-egg complexes were dispersed with hyaluronidase (Sigma). In order to assess the occurrence of fertilization, eggs were placed between slide and cover slip, and were examined using bright field microscopy (400X). Eggs were considered fertilized when a sperm tail was seen in the cytoplasm or in the perivitelline space. At P12, each implantation site was dissected, using a stereomicroscope, to verify the presence or absence of an embryo (60X). Only implantation sites containing live embryos are presented in the results.

2.11. Data analysis

Ovulation data are expressed as percentage of rats that ovulated calculated over the total number of animals treated and also as mean number of ovulated oocytes per ovulating rat ± SE.
All rats that mated had either fertilized eggs or implanted embryos. Thus, only the mean percentage of fertilized eggs and the mean number implanted embryos is presented per group. One-sided Fisher’s exact test or chi-square, as appropriate, was applied to two-way tables of percentage of rats that ovulated. Kruskal-Wallis test was used for mean number of ovulated oocytes or mean number of implanted embryos. Differences were considered statistically significant at p < 0.05. All statistical analyses were carried out on Intercooled Stata 6.0 computational program for Windows (Stata Corporation, College Station, TX, USA).

3. Results

3.1. Effect of preovulatory treatment upon ovulation (G1, G2 and G3)

Following injection of LNG or vehicle twice per day at both diestrus and proestrus (G1), the percentage of ovulating rats was 0% and 83%, respectively. The mean number of ovulated eggs in the control group was 8.0 ± 1.6 (Fig. 1). The percentage of ovulating rats among those treated once in the evening of diestrus (G2) or in the morning of proestrus (G3) was 25% and 63%, respectively, with LNG, and 98% in both control groups. The difference between experimental and control in G2 is statistically significant (1) = 0.02). The mean number of ovulated eggs was 6.0 ± 3.0 and 9.0 ± 2.0, respectively, for rats treated with LNG and 4.7 ± 0.8 and 7.1 ± 0.8 for controls. These differences were not statistically significant.

3.2. Effect of treatment before mating and ovulation upon fertilization and implantation (G4 and G5)

All rats in these experiments had either fertilized eggs on P2 or implanted embryos on P12. The mean percentage of fertilized eggs after injecting LNG or vehicle at 4:00 a.m. of proestrus and mating between 9:30 and 10:30 p.m. (G4), was 100% in both groups. Among those mated overnight (G5), the mean percentage of fertilized eggs was 100% in the LNG group and 98 – 1.8% in the control groups. The number of implanted embryos was 9.2 ± 1.1 and 10.2 -0.5, respectively (Fig. 2). None of these differences was statistically significant.

3.3. Effect of treatment after mating and before ovulation upon fertilization and implantation (G6 and G7)

All rats had either fertilized eggs on P2 or implanted embryos on P12. The mean percentage of fertilized eggs in rats treated at 8:00 a.m. of estrus and mated within the next hour was 75 ± 14.8% for LNG and 97 ± 3.3% for controls. The mean number of implanted embryos was 15.3 ± 0.6 and 12.9 ± 0.8, respectively (Fig. 2). None of these differences was statistically significant.

3.4. Effect of treatment after ovulation and before mating upon fertilization and implantation (G8)

The mean percentage of fertilized eggs in rats treated at 8:00 a.m. of estrus and mated within the next hour was 75 ± 14.8% for LNG and 97 ± 3.3% for controls. The mean number of implanted embryos was 15.3 ± 0.6 and 12.9 ± 0.8, respectively (Fig. 2). None of these differences was statistically significant.

3.5. Effect of treatment a after ovulation and mating upon and implantation (G9)

The mean percentage of fertilized eggs in rats injected immediately after mating in early estrus was 97 ± 3.3% for LNG and 100% for vehicle, and the mean number of implanted embryos was 11.8 ± 1.6 for LNG and 12.8 ± 0.8 for controls (Fig. 2). None of these differences was statistically significant.

3.6. Effect treatment after fertilization upon implantation (G10 and G11)

All rats had implanted embryos on P12. The mean number of implanted embryos in those treated at 11:00 p.m. of P1 and at 11:00 a.m. of P2 (G10) was 11.0 ± 0.8 for LNG and 10.6 ± 0.7 for controls. In those injected with LNG or vehicle at 11:00 p.m. of P3 and at 11:00 a.m. of P4 (G11), the mean number of implanted embryos was 11.8 ± 0.8 and 10.0 ± 10.9, respectively (Fig. 2). None of the differences was statistically significant.

4. Discussion

The results presented show that acute treatment with LNG, either shortly before or after mating, before or after fertilization, or before implantation, have no effect on the establishment of pregnancy in the intact rat (Fig. 2). This excludes that LNG interferes with postfertilization steps in this species and is in keeping with the fact that daily treatment of castrated rats with LNG is able to sustain pregnancy [4]. No evidence of antifertility effect attributable to interference with postfertilization events could be demonstrated. In contrast, preovulatory treatment with LNG was able to inhibit ovulation. The suppression of ovulation was heavily dependent upon the schedule of administration (Fig. 1).
Following injection of LNG either twice per day, once in the evening of diestrus or once in the morning of proestrus, the percentage of animals that failed to ovulate was 100%, 75% and 37%, respectively. The effectiveness of a single injection diminished as treatment became closer to the expected time of the ovulatory episode. Therefore, LNG inhibited ovulation totally or partially depending on the timing of treatment and possibly the total (lose administered. Tile dose-effect relationship was not adequately examined. The possibility that ovulation may have been delayed cannot be excluded, since we did not perform autopsies on the third day after estrus to test for tile occurrence of delayed ovulation.

Inhibition of ovulation in the rat after single administration of LNG has been documented previously. Ovulation was delayed in rats injected in the afternoon of metaestrus with LNG 50 l g/kg dissolved in oil, whereas it was totally suppressed by 200 l g/kg (5). When LNG dissolved in oil was injected in the afternoon of diestrus, it blocked ovulation in a dose-dependent fashion; the antiovulatory dose was 200-400 l g/kg (6). The most obvious differences with our experiments are the vehicle used and the time of administration. In our study, 50 l g/kg of LNG dissolved in 1,2-propanediol injected in the evening of diestrus inhibited ovulation in 75% of the animals and four doses administered 12 h apart beginning in the morning of diestrus (in total = 200 l g/kg), completely suppressed ovulation.

There is much information on the effects of LNG upon ovulation in women. In brief, LNG can inhibit ovulation when it is administered one or more days before follicular rupture [7-9]. It is known that LNG affects ovulation in animals [5,10] and women [11], at least through interference with signaling from the pituitary. Inconsistent effects of LNG upon endometrial markers of receptivity have been reported in women [8,9]. The effect of LNG upon markers of receptivity in the rat was not addressed in the present study.

In conclusion, postcoital administration of LNG at doses able to affect ovulation had no antifertility effect attributable to interference with postfertilization events in the rat.

Acknowledgments

Support for this study was provided by Fundación Instituto Milenio de Investigación en Biología Fundamental y Aplicada, Cátedra Presidencial en Ciencias, Fondecyt #898000-8 and RF98024#98.

References