Pharmacokinetics and endometrial tissue levels of levonorgestrel after administration of a single 1.5-mg dose by the oral and vaginal route

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Objective: To determine the pharmacokinetics and endometrial tissue levels of levonorgestrel when taken as a single dose of 1.5 mg either orally or vaginally by healthy women in the periovulatory phase of their menstrual cycle.

Design: Prospective randomized study.

Setting: Academic research institution.

Patient(s): Thirty women with regular cycles allocated to control (n = 5), oral (n = 13), and vaginal (n = 12) groups.

Intervention(s): Blood samples were drawn before (0 time) and at 0.5, 1, 2, 4, 6, 8, 24, 48, and 168 hours after levonorgestrel administration. Endometrial samples were collected 24 and 168 hours after levonorgestrel administration.

Main Outcome Measure(s): Plasma and endometrial tissue levels of levonorgestrel.

Result(s): Plasma concentrations of levonorgestrel were significantly greater during the first 48 hours after oral administration. However, 7 days after levonorgestrel administration, the plasma levels were similar for both treatments (3–5 nmol/L). Compared with vaginal administration, oral administration resulted in higher peak plasma concentrations (C_{max} 64 vs. 10.7 nmol/L), with a shorter time to reach the maximal concentrations (T_{max} 1.4 vs. 6.6 hours) and with a greater AUC (509 vs. 175 nmol/L). Interestingly, the half-life of levonorgestrel was shorter after oral administration (25 hours vs. 32.6 hours). Levonorgestrel tissue concentrations were not related to the plasma levels. Levonorgestrel values tended to be higher in endometrial tissue after vaginal administration. The ratio between plasma and endometrial concentrations of levonorgestrel differed significantly between the groups.

Conclusion(s): These data indicate that orally administered levonorgestrel achieves higher plasma levels sooner than vaginally administered levonorgestrel. However, plasma levels after vaginal administration are more sustained and were likely to be sufficient for ovarian suppression. Therefore, the vaginally administered levonorgestrel could be considered as an alternative option for emergency contraception.

Key Words: Levonorgestrel, pharmacokinetics, oral and vaginal route

Levonorgestrel is a synthetic progestin used as a progestin-only emergency contraceptive (EC) for women who have had unprotected intercourse, who have been sexually assaulted, or who have reason to believe that their contraceptive method has failed. Levonorgestrel is administered orally, either in two doses of 0.75 mg, given 12 hours apart, or in a single dose of 1.5 mg as soon as possible within 72 hours of intercourse (1, 2).

Pharmacokinetic information exists for two doses of 0.75 mg of levonorgestrel, 12 hours apart, for EC use (3–5). The oral bioavailability of levonorgestrel is >90% but has been reported to depend on the dosage form and to be affected by a concomitant administration of estrogens (6, 7). The maximum concentration after a single oral dose of 0.75 mg of levonorgestrel is reached 1 hour after administration, with a half-life ranging from 20 to 60 hours (3–5). However, there is limited information on the pharmacokinetics of a single dose of 1.5 mg of levonorgestrel (two tablets) in fertile women during the periovulatory period of the menstrual cycle (3). Therefore, the present recommendation for this treatment, including dose and time of administration, is based on clinical experience and not on pharmacokinetic data.

In clinical settings, vaginal administration of progestin formulations in vaginal rings, suppositories, or tablets has

been highly efficient for contraception or for progesterone (P) supplementation in luteal phase defect or in assisted reproduction (8, 9).

The purpose of this study was to characterize the pharmacokinetics of a single dose of 1.5 mg of levonorgestrel (two tablets of 0.75 mg each) when administered orally or vaginally during the periovulatory period of the normal menstrual cycle. Furthermore, the endometrial tissue levels of levonorgestrel were determined 24 and 168 hours after oral and vaginal administration to see whether the response differed at the tissue level.

MATERIALS AND METHODS
Subjects

The experimental protocol was approved by the institutional review board of the Hospital San Borja-Arriarán Santiago, Santiago, Chile, and signed informed consent was obtained from each woman who agreed to participate in the study after the study was completely explained to her. All participants were healthy, aged 24 to 43 years, with a body mass index (BMI) ranging from 17 to 32 kg/m^2, with regular menstrual cycles, and had undergone tubal ligation ≥ 6 months before participating in the study to avoid the risk of pregnancy. None had received any hormonal therapy for the last 3 months. Before enrollment and at the end of the study, each subject underwent physical examination and clinical laboratory tests, including blood chemistry, hematology, and liver enzymes.

Study Design

The day of admission to the study was close to day 10 of the menstrual cycle. Vaginal ultrasound was performed, including measurements of the follicular diameter and endometrial thickness. When the leading follicle achieved a diameter of >16 mm and urinary LH was detected (Clearplan; Unipath, Bedford, UK), two tablets of

levonorgestrel (0.75 mg each; Postinor II; Gedeon Ritcher Ltd., Budapest, Hungary) were administered as a single dose either orally (n = 13) or vaginally (n = 12) according to randomization. Five women in the control group received placebo tablets of levonorgestrel. Blood samples (5 mL) were taken before administration of the tablets (time 0) and then serially at 0.5, 1, 2, 4, 6, 8, 24, 48, and 164 hours after the treatment. One of us (A.E.) administered the tablets vaginally and withdrew the blood samples. The participants remained in the clinic for 8 hours. Endometrial biopsies were taken with Pipelle (Pipelle de Gornier Laboratoire CCD, Paris, France) without anesthesia during separate visits. The biopsies were obtained for 11 women at 24 hours and for all the individuals at 7 days after the treatment. A small portion of the endometrial tissue was fixed immediately in formaldehyde buffer solution for endometrial dating. The large portion was weighed and frozen without delay in dry ice and was kept at $-80°C$ for levonorgestrel determination.

Levonorgestrel Assay

The concentration of plasma levonorgestrel was determined by RIA. The method was described by Weiner and Johansson (10). Briefly, duplicate sample volumes of 0.2 mL were extracted with diethyl ether and evaporated to dryness in a stream of nitrogen. The residues were then incubated with the specific antibody (Schering AG, Berlin, Germany) and tracer [14, 15-^{3}H]-levonorgestrel overnight at 4°C to obtain the equilibrium. Bound and unbound ligands were separated by utilization of dextran-coated charcoal.

The sensitivity of the assay was set to 0.2 pmol/L (50 pg/mL). The intra-assay coefficient of variation (CV) was 6%, and the interassay CV, between 8% and 12%.

Levonorgestrel concentration in endometrial biopsies was determined by RIA according to the method of Nilsson et al. (11). The tissue samples were thawed, weighed, and homog-

	TABLE 1		
Clinical and endocrine characteristics of subjects.			
Parameter	Vaginal administration	Oral administration	Control
Subjects (n)	12	13	5
Age (y)	36.5 ± 1.0	36.7 ± 1.3	33.4 ± 2.6
BMI (kg/m^2)	27.9 ± 0.8	24.7 ± 1.0	26.9 ± 2.6
Menstrual interval (d)	28.2 ± 0.1	27.6 ± 0.7	28.0 ± 0.1
LH (IU/L)	20.3 ± 6.7	16.9 ± 4.8	22.2 ± 14.5
Progesterone (ng/mL)	1.2 ± 0.31	0.87 ± 0.27	1.7 ± 0.65
Estradiol (pg/mL)	143 ± 15.9	136 ± 13.2	172 ± 51.2
Follicular diameter (mm)	17.5 ± 0.4	18.0 ± 0.8	18.1 ± 0.2

Note: Data are mean ± SEM. *P* was not significant for any comparison.

Devoto. Oral and vaginal administration of levonorgestrel. Fertil Steril 2005.

enized in ice-cold distilled water. Duplicate 1-mL samples of the homogenate were extracted twice with diethyl ether and evaporated to dryness in a stream of nitrogen. The residues were dissolved in 1 mL of assay buffer containing 5% of methanol. Antibody and tracer were added to duplicate 0.1-mL volumes of redissolved material. Samples were treated thereafter as described above in RIA for plasma samples. Protein determinations of the homogenates were performed by using Uptima commercial kit (BC Assay Protein Quantitation Trial Kit; Interchim, Montlucon, France). The intra-assay CV was 10%, and the interassay CV, between 11% and 17%.

Pharmacokinetic and Statistical Analysis

Each individual concentration–time curve was fitted. The area under the concentration–time curve, maximal concentration (C_{max}), time to reach maximal concentration (T_{max}), and biological half-life were obtained for each route of administration. The normal distribution of data was tested using Kolmogorov-Smirnov test. Analysis of variance for more than two groups was applied to normally distributed data followed by t test and Mann-Whitney and Kruskal Wallis test for independent nonparametric variables.

RESULTS

The clinical and endocrine characteristics of the women are summarized in Table 1. These data indicate the clinical and endocrine homogeneity of the participants.

Figure 1 illustrates the plasma concentrations of levonorgestrel as function of time. Individual levonorgestrel pharmacokinetic profiles are presented in Figure 1A and 1B for the oral and vaginal routes, respectively. All plasma assays performed for levonorgestrel on control women demonstrated undetectable levels.

The comparison of mean levels of levonorgestrel between the vaginal and oral route of administration is summarized in Table 2. The oral route of administration resulted in greater plasma concentrations of levonorgestrel throughout the study period as compared with the vaginal route. Oral administration appeared to have a better solubility and a faster absorption compared with vaginal administration because the peak values were achieved sooner (1 hours to 4 hours after oral levonorgestrel administration vs. 6 hours to 8 hours for vaginal administration) and were sixfold higher (C_{max} 64 vs. 10.7 nmol/L). The levonorgestrel plasma concentrations remained significantly higher at 24 hours after oral administration (11.0 $\pm$ 2.2 nmol/L), as compared with the plasma levels achieved at 24 hours for the vaginal route (5.7 $\pm$ 0.6 nmol/L). The AUC calculated for 24 hours after levonorgestrel administration was significantly higher for the oral route, which presumably reflects the higher levonorgestrel concentration noted in this group. Seven days after administration, levonorgestrel concentrations exhibited similar levels (2.0–3.0 nmol/L) in the oral and vaginal groups, respectively.

Levonorgestrel plasma levels in women after (**A**) oral (n = 13) and (**B**) vaginal (n = 12) administration of levonorgestrel (1.5 mg) during the periovulatory period of the menstrual cycle. Individual concentrations data are plotted vs. time.
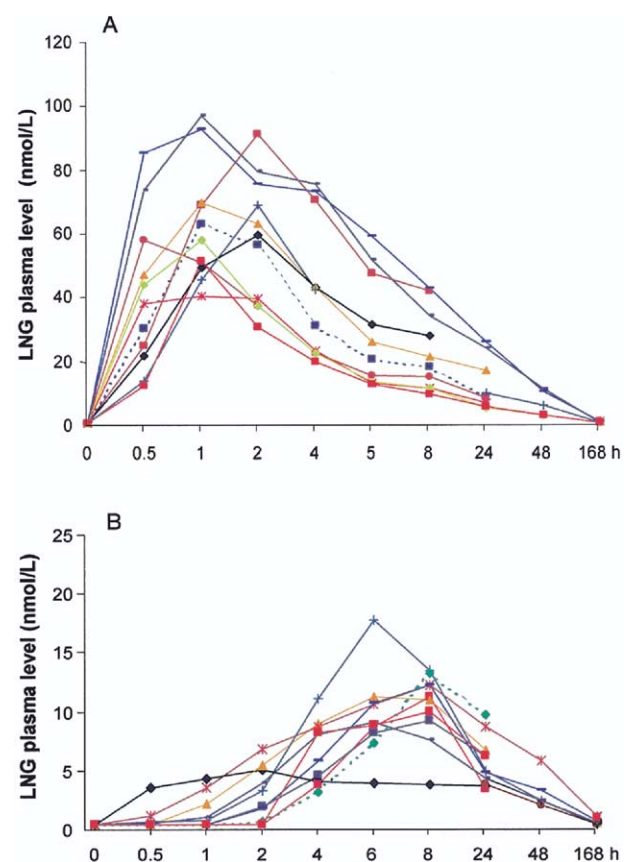
Devoto. Oral and vaginal administration of levonorgestrel. Fertil Steril 2005.

Endometrial tissue concentrations (nanomoles per milligram of tissue) at 24 hours and 168 hours of levonorgestrel administration are presented in Figure 2. Endometrial samples were collected for the same individual at 24 hours and 168 hours, but we only obtained two endometrial biopsies from five women in the oral group and from six who received vaginal levonorgestrel administration. The tissue concentrations of both groups exhibited a progressive decline throughout the study period, achieving similar level at 168 hours. The tissue concentrations of levonorgestrel for subjects in the vaginal-route group displayed a trend to exhibit higher endometrial tissue concentrations at 24 hours compared with the oral-route group. All endometrial assays performed for levonorgestrel on control subjects demonstrated undetectable levels.

Figure 3 depicts the ratios between plasma levonorgestrel at the time of C_{max} (nmol/L) and endometrial levonorgestrel levels (nmol/L) at 24 hours for both types of treatment.

The ratio was calculated on the basis of individual plasma and tissue levonorgestrel concentrations. The ratio was significantly different for the vaginal route of levonorgestrel administration.

DISCUSSION

Levonorgestrel is currently used for progestin-only EC formulation. Two tablets of levonorgestrel (0.75 mg each) are administered orally, either 12 hours apart or as a single dose of 1.5 mg. These treatments are effective and have low incidence of side effects (1, 2). However, the pharmacokinetic data on these regimens are limited, particularly on the single oral dose of 1.5 mg (3). A review of the literature indicates that other studies had previously confirmed the effectiveness of the vaginal route for progestin administration, including oral capsule of micronized P used for luteal-phase supplementation and levonorgestrel in the form of

contraceptive pills (0.5 mg of levonorgestrel and 0.05 mg of ethinyl estradiol) or the vaginal ring containing levonorgestrel for contraception, respectively (6, 8, 9). We hypothesized that the vaginal route could represent an alternative option for delivering levonorgestrel tablets as EC. The aim of the present investigation was to characterize pharmacokinetic patterns of two different routes of administration (oral and vaginal) of levonorgestrel tablets in a single dose of 1.5 mg (two 0.75-mg tablets). Thus, the present study extends the knowledge of pharmacokinetics of levonorgestrel and includes a simultaneous appraisal of levonorgestrel concentrations within the endometrial tissue.

Large intersubject variations of plasma levonorgestrel concentrations in relation to the dose have been noted (7). In addition, BMI, the level of sex hormone–binding globulin, and administration of estrogen can affect levonorgestrel bio-

FIGURE 2

Endometrial tissue level of levonorgestrel from participants that have two endometrial specimens at 24 hours and 168 hours after treatment. Oral administration (n = 5) and vaginal administration (n = 6). Individual endometrial concentrations data are plotted vs. times. *Broken lines* indicate oral administration; *solid lines* indicate vaginal administration.
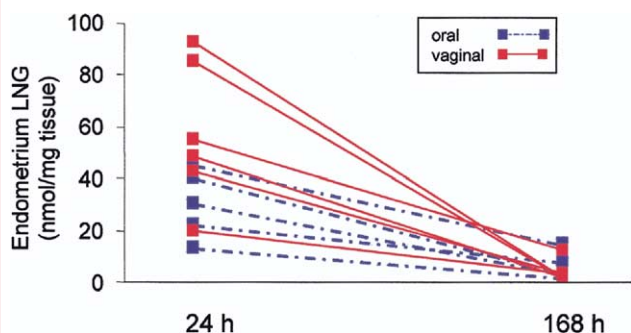
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FIGURE 3

Ratio between plasma levonorgestrel C_{max} (nmol/L) and endometrial levonorgestrel levels (nmol per mg tissue) at 24 hours. Values represent mean concentrations, and error bars are the 95% confidence interval. [a]$P<.05$ between treatments.
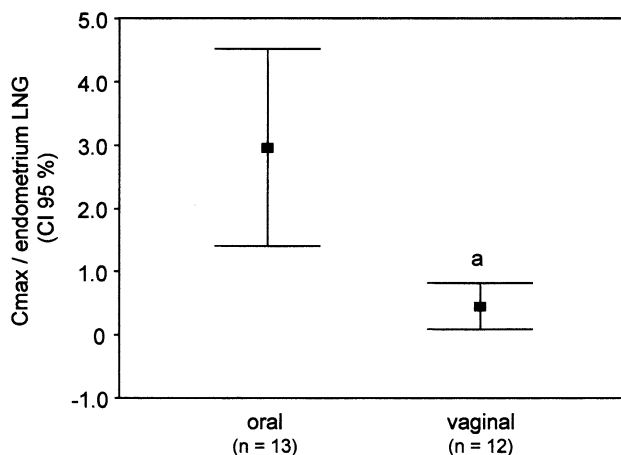
Devoto. Oral and vaginal administration of levonorgestrel. Fertil Steril 2005.

availability. To in part reduce this complexity, specific inclusion criteria were established in the present study. All the participants of this randomized clinical trial exhibited similar clinical features. Levonorgestrel was administered in the late follicular phase, and blood samples for levonorgestrel determination were obtained at varying times after levonorgestrel administration.

Our data confirm the findings of Johansson et al. (3), which indicated large intersubject variations in plasma concentrations when levonorgestrel was administered orally. These data are similar, with the exception that we observed a higher C_{max} (64 nmol/L vs. 39.3 nmol/L), and it was reached sooner (95 minutes vs. 150 minutes). Interestingly, the AUC was similar in our study. These differences between our data and those of Johansson et al. (3) may be explained in terms of different study design, number of participants, inclusion criteria, and ethnic differences.

In contrast, our data demonstrate that when levonorgestrel was administrated vaginally, the intersubject variations in plasma concentrations of levonorgestrel were smaller than those after the oral route. Interestingly, plasma C_{max} (64 nmol/L) and the AUC (509 nmol/L per hour) during 24 hours for the orally administered levonorgestrel were greater than those achieved by the vaginal route (sixfold and twofold), respectively. In addition, the vaginal route exhibited a significant delay in reaching T_{max} (6.6 hours), as compared with the oral route (1.4 hours). However, the half-life of vaginally administered levonorgestrel (32.6 hours) was greater than that of orally administered levonorgestrel (25.0 hours). These findings may suggest that dissolution, absorption rate of levonorgestrel pills, and the first-pass metabolism through the liver of the levonorgestrel is dependent on the route of administration.

To our knowledge, this is the first report of a simultaneous assessment of levonorgestrel in the plasma and the endometrium of women treated with levonorgestrel in doses as used for EC. Previous report had indicated that vaginally administered micronized P maximimizes tissue levels with minimal side effect (9). Our findings indicate that levonorgestrel tissue concentrations exhibited a progressive decline throughout the study period, achieving similar levels at 168 hours. Interestingly, the tissue concentrations of levonorgestrel for subjects in the vaginal group displayed a trend to exhibit higher endometrial tissue concentrations at 24 hours compared with the oral group. However, this difference did not reach statistical significance, presumably because of notable intersubject variability. Interestingly, the ratio between individual plasma concentrations (C_{max}) and endometrial tissue concentrations of levonorgestrel at 24 hours after administration was close to 1 and was significantly different for the vaginal group. These data indicate that vaginally administered levonorgestrel is efficient in targeting the uterus and presumably reflect a preferential uptake of the progestin administered by this route. It has been postulated elsewhere that the endometrium responds to a critical plasma level of P that is not reflected in the peripheral plasma level (9). If this is the case for levonorgestrel, then P receptor, androgen receptor, and endometrial sex hormone–binding globulin, which are normally expressed by the human endometrial tissue and which bind levonorgestrel with high affinity, may play a crucial role in the regulation of local endometrial levonorgestrel concentrations (12–14).

Our present results, particularly those related to the high level of plasma levonorgestrel after oral administration and the limited binding capacity of the endometrium for levonorgestrel, support the Johansson et al. (3) statement regarding the real need for high levonorgestrel plasma levels in EC treatments. Furthermore, the prevailing notion is that levonorgestrel plasma concentrations modify ovarian function with levels significantly lower than those observed in the present study (15).

In conclusion, we have documented the pharmacokinetic parameters and endometrial tissue levels of levonorgestrel after oral or vaginal administration of a single dose of two tablets, each containing 0.75 mg of levonorgestrel. The pharmacokinetic study supports differential patient responses to the treatments, which may reflect difference in absorption rate and metabolism between routes of administration (16). Vaginal administration results in lower plasma levels of levonorgestrel as compared with the oral route. However, plasma levels attained after vaginal administration are high enough to restrain ovulation. In addition, previous studies have indicated that levonorgestrel administered in vivo or to an in vitro system has the capability to reduce sperm migration and spermatozoa–ovum fusion (17–19). Thus, the pharmacokinetic features and presumably the relatively high local concentrations of levonorgestrel achieved in the endometrium when using the vaginal route may indicate that levonorgestrel also could be administered vaginally when used for emergency contraception.

REFERENCES

1. Task Force on Post-ovulatory Methods of Fertility Regulation. Randomized controlled trial of levonorgestrel versus the Yuzpe regimen of combined oral contraceptives for emergency contraception. Lancet 1998;352:428–33.
2. von Hertzen H, Piaggio G, Ding J, Chen J, Song S, Bartfai G, et al. Low dose mifepristone and two regimens of levonorgestrel for emergency contraception: a WHO multicentre randomised trial. Lancet 2002;360: 1803–10.
3. Johansson E, Brache V, Alvarez F, et al. Pharmacokinetic study of different dosing regimens of levonorgestrel for emergency contraception in healthy women. Hum Reprod 2002;17:1472–6.
4. Kook K, Gabelnick H, Duncan G. Pharmacokinetics of levonorgestrel 0.75 mg tablets. Contraception 2002;66:73–6.

5. Tremblay D, Gainer E, Ulmann A. The pharmacokinetics of 750 μg levonorgestrel after administration of one single dose or two doses at 12- or 24-h interval. Contraception 2002;64:327–31.

6. Back DJ, Grimmer SFM, Rogers S, Stevenson PJ, Orme ML. Comparative pharmacokinetics of levonorgestrel and ethinylestradiol after intravenous, oral, and vaginal administration. Contraception 1987;36:471–9.

7. Fotherby K. Levonorgestrel: clinical pharmocokinetics. Clin Pharmacol 1995;28:203–15.

8. Alvarez F, Faundes A, Johansson E, Coutinho E. Blood levels of levonorgestrel in women following vaginal placement of contraceptive pills. Fertil Steril 1983;40:120–3.

9. Miles R, Paulson R, Lobo R, Press M, Dahmoush L, Sauer M. Pharmacokinetics and endometrial tissue levels of progesterone after administration by intramuscular and vaginal routes: a comparative study. Fertil Steril 1994;62:485–90.

10. Weiner E, Johansson EDB. Plasma levels of d-norgestrel, estradiol and progesterone during treatment with silastic implants containing d-norgestrel. Contraception 1976;14:18–92.

11. Nilsson CG, Haukkamaa M, Vierola H, Luukkainen T. Tissue concentrations of levonorgestrel in women using a levonorgestrel-releasing IUD. Clin Endocrinol 1982;17:529–36.

12. Juchem M, Pollow K. Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor. Am J Obstet Gynecol 1990; 163:2171–83.

13. Misao R, Itoh N, Mori H, Fujimoto J, Tamaya T. Sex hormone-binding globulin mRNA levels in human uterine endometrium. Eur J Endocrinol 1994;131:623–9.

14. Mertens HJ, Heineman MJ, Koudstaal J, Theunissen P, Evers JL. Androgen receptor content in human endometrium. Eur J Obstet Gynaecol Reprod Biol 1996;70:3–11.

15. Brache V, Blumenthal PD, Alvarez F, Dunson TR, Cochon L, Faundes A. Timing of onset of contraceptive effectiveness in Norplant Implant users II. Effect on the ovarian function in the first cycle of use. Contraception 1999;59:245–51.

16. Humpel M, Wendt H, Pommerenke G, Weib C, Speck U. Investigation of pharmacokinetics of levonorgestrel to specific consideration of a possible first-pass effect in women. Contraception 1978;17:207–20.

17. Kesseru E, Garmendia F, Wesrphal N, Parada J. The hormonal and peripheral effects of d-norgestrel in postcoital contraception. Contraception 1974;10:411–24.

18. Yeung WSB, Chiu PCN, Wang CH, Yao YQ, Ho PC. The effects of levonorgestrel on various sperm functions. Contraception 2002;66: 453–7.

19. Croxatto HB, Devoto L, Durand M, Ezcurra E, Larrea F, Nagle C, et al. Mechanism of action of hormonal preparations used for emergency contraception: a review of the literature. Contraception 2001;63:111–21.