J. steroid Biochem. Vol. 27, No. 4–6, pp. 1003–1007, 1987
Printed in Great Britain.

0022-4731/87 $3.00 + 0.00

Proceedings of the VII International Congress on Hormonal Steroids (Madrid, Spain, 1986)

NORETHISTERONE CONTRACEPTIVE MICROSPHERES

ROBERTO RIVERA, GLORIA ALVARADO, CONSUELO FLORES SILVIA ALDABA and ANA HERNANDEZ

Instituto de Investigacion Cientifica, Universidad Juarez del Estado de Durango, Avenida Universidad y Fanny Anitua Apartado Postal B-7, Durango, Dgo., Mexico

Summary—Two formulations of polylactic and polyglycolic acid microcapsules containing 75 and 100 mg of NET respectively were studied for a 90-day period of anticipated contraceptive effect in two groups of five women. A 200 mg dose of NET preparation was also studied for a 180-day period of anticipated contraceptive effect in 19 women. Alteration in menstrual cycle, with tendency to short bleeding episodes, spotting days, and amenorrhea were the most important collateral effects. In the majority of cases, ovulation was inhibited. No cases of pregnancy were presented. The obtained NET circulating levels were very stable during the period of anticipated contraceptive effect.

INTRODUCTION

The main objectives in the development of new methods for fertility regulation have been:

1. to reduce the incidence of side-effects associated with the use of hormonal contraceptives;
2. to obtain the most effective method;
3. to be culturally acceptable;
4. to avoid interference with lactation;
5. to be reversible;
6. to be completely independent of coital activity; and
7. to be able to be administered by non-medical personnel [1].

This has brought about the design of systems that release contraceptive steroids gradually, due to the biochemical or pharmacological characteristics that are inherent in the molecules or in the constitution of the vehicle, and to the interaction that the steroids exert on the tissues [2].

The advantages obtained from the use of long-lasting contraceptive methods are:

1. Highly effective contraception, superior to that of oral hormones or that of intrauterine devices. In addition, the theoretical and actual effectiveness of these systems are very similar.
2. Greater safety due to an important decrease of side-effects, particularly the serious risks attributable to estrogens. This is due to the fact that a majority of the systems base their contraceptive effect on progestins.
3. Wide acceptability explained by the minimal participation or effort required of the couple to use the method.
4. They do not interfere with lactation.
5. Ease of use, since they require isolated motivation.
6. The release of the steroid is stable, owing to the constitution of the delivery system. The circulating levels that may be obtained by the system during the period of effectiveness are very uniform, and close to the limits of zero-release. This last point can be observed in Fig. 1, where hypothetical levels of a programmed-release steroid are compared with the levels of an oral contraceptive.

The levels of minimum intervention occur when the steroid is maintained within the range of therapeutic dosage. The administration of oral contraceptive introduces an immediate elevation of hormonal levels which diminishes with time; therefore, repeated dosages must be administered at frequent intervals in order to maintain these levels

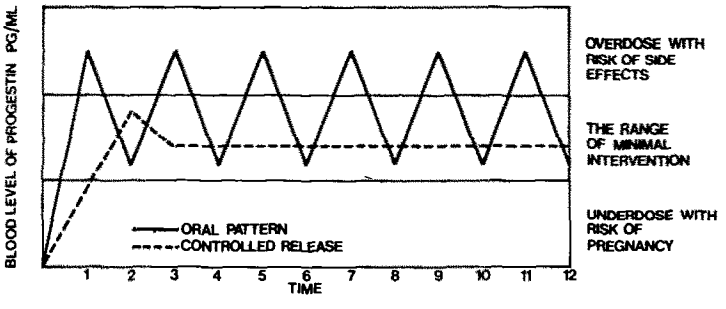

Fig. 1. Blood levels of a hypothetical steroid following either oral administration or programmed delivery.

within effective limits. These hormonal fluctuations may range into overdosage, with the appearance of side-effects, or into the zone of insufficient dosage, with the risk of pregnancy. On the other hand, the programmed-release systems do not have these fluctuations and are maintained within the range of minimum intervention during the entire period of their effectiveness [3, 4].

Of the systemic release long-acting contraceptive methods, the injection of several hormones as microcapsules is now being pursued. Microcapsules are spherical particles made of biodegradable polymers such as polyglycolic or polylactic acid containing micronized crystals of NET dispersed in an homogeneous form, and suspended in a medium of carboxymethylcellulose or EDTA.

The NET microcapsules are administered by i.m. injection, and the duration of its contraceptive effect depends mostly on the amount of the steroid and on the size of the microspheres.

NORETHISTERONE MICROSPHERES 6-MONTH SYSTEM

Our purpose in the study of this system was to determine: the safety of the procedure by measuring the side-effects associated with the use of the system; the effects on ovarian function assessed by determinations of estradiol and progesterone; the characteristics of the bleeding patterns; the effectiveness of the method measured by the incidence of pregnancy, and the type of NET levels obtained by the system.

METHODOLOGY

Nineteen healthy women volunteers, who were selected according to criteria for the use of hormonal contraceptives, received an i.m. injection of 800 mg of microspheres of polylactic acid containing 200 mg of NET, with an anticipated contraceptive effect of 6 months. The microspheres were suspended in a carboxymethylcellulose medium, and shaken vigorously in a Vortex mixer for 3 min immediately before injection.

The subjects had a control cycle and an 8-month observation period after injection. Throughout this interval, the volunteers kept a careful record of their menstrual cycles. On the completion of the study period, the menstrual events were analyzed in accordance with the procedure described by Rodriguez *et al.*[5].

Weekly samples of venous blood were obtained for the determination of estradiol, progesterone and NET. These hormones were analyzed by means of specific radioimmunoassays using WHO-HRP methodologies [6].

RESULTS

No problems arose from the injection of the system. However, it was necessary to inject the material immediately after shaking, otherwise sedimentation of the microcapsules would occur, and the efficiency of the injection would diminish. The procedure caused slightly more discomfort than that noted with other injection, such as Depo Provera, and more similar to that observed with the injection of benzatinic penicilline. No local reaction appeared immediately after the injection, nor did it appear during the 8-month observation period.

Effects on ovarian function. In Fig. 2 the serum levels of NET, estradiol and progesterone are presented. It can be observed that NET levels during the first 5 weeks ranged from 2 to 3 ng/ml, and until the 15th week a level above 1.0 mg/ml was maintained, with levels below 0.5 ng/ml up to the 25th week. Beyond this time, the NET levels were very low and nondetectable values were frequently obtained. Generally, very stable levels of NET with a gradual decline were observed in all the women throughout the study period.

The whole group estradiol serum levels were below 100 pg/ml during most of the anticipated period of effect, suggesting inhibition of follicular activity. One subject showed an elevation in the estradiol levels in the 16th week, up to 227 pg/ml. Another four subjects also had moderate rises of estradiol in weeks 22–24, suggestive of a return of follicular activity within the anticipated period of contraceptive effect.

The levels of progesterone that are shown were

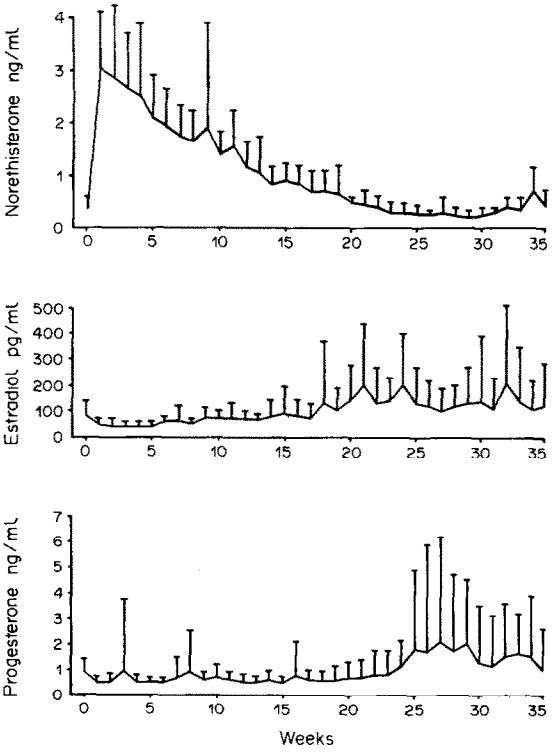

Fig. 2. Mean levels of norethisterone, estradiol and progesterone in 19 women using a 6-month contraceptive preparation with 200 mg of NET microspheres.

Table 1. Menstrual effects per 30-day reference period in 19 women using a 6-month contraceptive preparation with 200 mg of NET microspheres

	1* 30	31 60	61 90	91 120	121 150	151 180	181 210	211 240
Nonbleeding	0	1	5	9	5	3	3	2
Bleeding and spotting 1–7 days	13	10	8	4	7	10	14	16
Bleeding and spotting 8–30 days	6	8	6	6	7	6	2	1

*Period of injection.

clearly indicative of non-ovulation in the majority of the cases, throughout the 24 weeks of contraceptive effect. In four subjects ovulatory peaks were shown in weeks 4, 7, 17 and 23 (range of 4.2–5.9 ng/ml) followed by menstration, a pattern suggestive of ovulatory cycles within the period of anticipated contraceptive effect.

Side-effects. The most important problem associated with the use of the system was the alteration in the menstrual pattern, basically amenorrhea and prolonged menstrual bleeding. Dizziness and nausea, and headache appeared in 6 and 5 women respectively.

Effects on the menstrual pattern. There were important disruptions of the menstrual pattern in most of the cases ranging from frequent bleeding or spotting to prolonged amenorrhea.

Nine of the 19 subjects showed prolonged non-bleeding intervals of 60–149 days. These intervals were most likely to occur 3 months after the injection. Upon completion of the 8-month observation period, two of the subjects were still amenorrheic (Table 1).

In the control cycle previous to the injection, 13 cases presented bleeding and spotting for 1–7 days. However, following the injection, most of the cases that were not amenorrheic had long periods of bleeding or spotting which disappeared by the end of the observation period. In eight cases prolonged bleeding and spotting days ranging from 8 to 30 days occurred during the anticipated period of contraceptive effect. The menstrual disruption persisted only in one case by the end of the observation period.

Contraceptive effectiveness. No pregnancies occurred during the 24 weeks of the study, due in most cases to the inhibition of ovarian activity. In the other cases, in which ovarian function was maintained, it was most probably due to the effect that progestins have on the endometrium or on the cervical mucus.

Return of fertility. Twelve of the subjects ovulated before the 35th week of the injection. The remaining seven cases had not yet ovulated by the end of this period.

DISCUSSION

The injection of the system was not a difficult procedure; and non-medical personnel can apply the injection. However, vigorous shaking was required in order to achieve homogeneous suspension of the microspheres and good efficiency of the injection.

With the exception of the presence of dizziness and nausea in 35% and headache in 26% of the subjects, the side-effects were not important. The main problem was the alteration in the menstrual cycle, with a general tendency towards the gradual diminution of the bleeding episodes, the appearance of numerous days of spotting, and the presence of amenorrhea. According to our experience, women tend to tolerate better alterations in the menstrual pattern associated with the use of contraceptive methods when they are informed that these alterations are likely to occur. Several studies have reported that the problems concerning the menstrual cycle are one of the main reasons for discontinuing long-term injectables [7].

No pregnancies occurred during the 24 weeks of the anticipated contraceptive effect.

The levels of NET obtained with the system were very stable throughout the study period, and definitely better than those found with NET-EN [8]. No important variations were observed among the cases or within the women themselves.

It has been reported that circulating levels of NET above 1.0 ng/ml are necessary to inhibit ovulation. In the subjects who ovulated during the period of anticipated contraceptive effect, the levels of NET were found to be between 0.5 and 1.0 ng/ml. After week 20 the levels of NET were very low. In order to ensure the suppression of ovarian activity throughout a 6-month period it seems necessary to use a system with a dosage greater than 200 mg of NET.

NORETHISTERONE MICROSPHERES 3-MONTH SYSTEM

The study of this system was undertaken with the purpose as previously mentioned for the 180-day system.

METHODOLOGY

In order to carry out this study, an i.m. injection of polylactic and polyglycolic acid microspheres, containing 75 or 100 mg of NET was applied to two groups of five women each, anticipating a period of contraceptive effect of 90 days. The subjects had a control cycle and an observation period of 5 months after the injection.

RESULTS

In some subjects local discomfort appeared after the injection, but it disappeared within the first 24 h without requiring any treatment.

Effects on ovarian function. The serum of NET, estradiol and progesterone in the subjects who received 75 mg of NET are shown in Fig. 3.

The values of NET remained between 1.0 and 2.0 ng/ml in the first 5 weeks, between 0.5 and 1.0 ng/ml until the 10th week, and below 0.5 until the 15th week. The NET levels were nondetectable beyond the 25th week.

In the whole group the levels of estradiol were very low, as those observed in early follicular stages, suggesting the suppression of follicular maturation.

In the majority of the cases the serum progesterone levels were non-ovulatory throughout the 90 days of anticipated contraceptive effect. One subject had a progesterone level in the 17th week of 5.8 ng/ml, becoming the earliest ovulation observed during the study.

The hormonal values of NET, estradiol and progesterone found in those subjects who received 100 mg of NET are shown in Fig. 4. The NET levels were between 1.0 and 3.0 ng/ml in the first 15 weeks, and gradually diminished until they were nondetectable at the end of week 23.

This curve with an initial elevation in the values of NET seems to be caused by the diffusion of the steroid from the microspheres. It was followed by a gradual fall with the appearance of a second peak caused by the biodegradation of the vehicle [9].

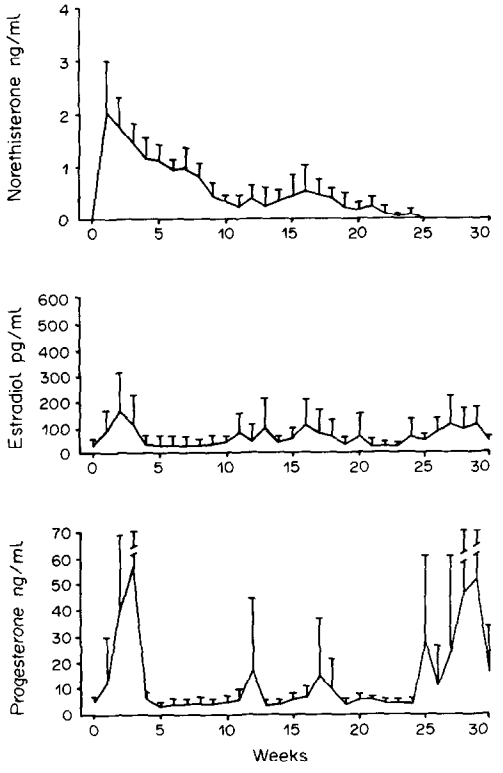

Fig. 3. Mean levels of norethisterone, estradiol and progesterone in five women using a 3-month contraceptive preparation with 75 mg of NET microspheres.

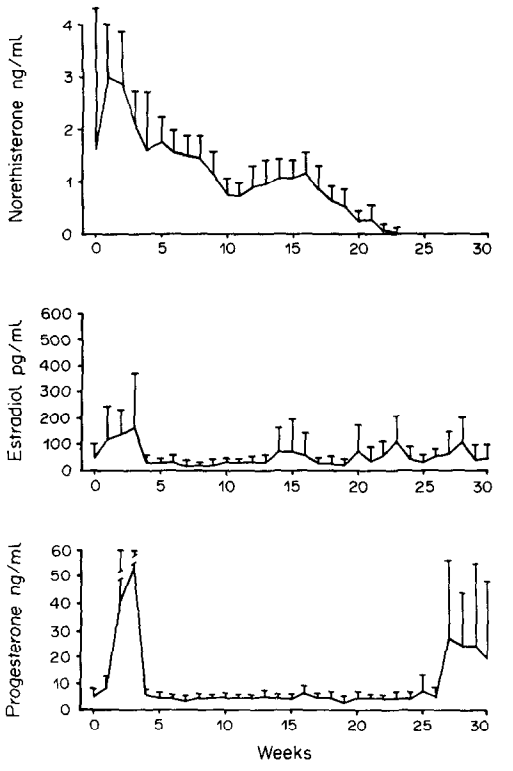

Fig. 4. Mean levels of norethisterone, estradiol and progesterone in five women using a 3-month contraceptive preparation with 100 mg of NET microspheres.

The values of estradiol were below 100 pg/ml in the 90-day period after the injection.

The serum levels of progesterone were clearly indicative of ovarian activity inhibition with the contraceptive effect period.

Side-effects. No important collateral effects appeared. The reported discomfort was related to the alteration of the menstrual cycle.

Effects on the menstrual cycle. The effects upon the menstrual cycle are presented in Table 2. The upper section shows the results obtained in the women who received 75 mg of NET; the lower section illustrates those subjects who received 100 mg.

In two cases where 75 mg of NET was used, there was a high frequency of prolonged cycles with an appearance of amenorrhea that lasted from 90 to 108 days. By the end of the observation period one case was still amenorrheic. On the other hand, prolonged bleeding or spotting was also a common observation. Prolonged menstrual bleeding with a duration of 8–23 days occurred in two subjects (Table 2).

In the group of women who used 100 mg of NET, amenorrhea appeared in three cases, lasting between 104 and 184 days. This effect disappeared in the first control month after the 90 days of the contraceptive period (Table 2).

Table 2. Menstrual effects per 30-day reference period in two groups of five women each, using a 3-month contraceptive preparation with 75 and 100 mg of NET microspheres respectively

	1* 30	31 60	61 90	91 120	121 150	151 180	181 210
Nonbleeding	0	0	2	2	1	2	1
Bleeding and spotting 1–7 days	4	3	2	1	2	1	4
Bleeding and spotting 8–23 days	1	2	1	2	1	2	0
Nonbleeding	0	0	2	3	3	0	3
Bleeding and spotting 1–7 days	5	3	3	1	1	4	2
Bleeding and spotting 8–18 days	0	2	0	1	1	1	0

*Period of injection.

Contraceptive effectiveness. No pregnancies occurred during the period of contraceptive effect, apparently due to the inhibition of ovulation.

Return of fertility. There was no difference in the return of ovulation between the subjects receiving 75 or 100 mg of NET. Ovulation recurred at an average of 22 weeks after the injection. In one case where 75 mg were used, and in two cases who received 100 mg of NET, ovulation had not occurred 30 weeks after the injection.

DISCUSSION

The levels of NET obtained with the dose of 75 mg of NET fluctuated between 1.0 and 2.0 ng/ml up to the 5th week, and between 0.5 and 1.0 ng/ml until the 10th week, with a gradual fall to nondetectable levels 23 weeks after the injection. These values were not sufficient to suppress ovarian activity in all cases. The levels of NET that were achieved with the injection of 100 mg of NET were above 1.0 ng/ml until the 9th week and between 0.5 and 1.0 ng/ml until the 19th week. Around the 23rd week the levels were not detectable. Ovulation was inhibited in all cases.

No important side-effects were present. The alterations in the menstrual cycle with the decrease in bleeding and the beginning of amenorrhea was more frequent with the 100-mg dosage of NET. No pregnancies occurred during the period of anticipated contraceptive effect with both doses, due to the inhibition of ovulation. The method was effective, safe and well accepted.

REFERENCES

1. Beck R. L., Cowsar D. and Pope Z. V.: Long-acting steroidal contraceptive systems. In *Research Frontiers in Fertility Regulation* Vol. 1 (1980) pp. 1–3.
2. Howard G., Warren R. J. and Fotherby K.: Plasma levels of norethisterone in women receiving norethisterone oenanthate intramuscularly. *Contraception* **12** (1975) 45–52.
3. Nygren K., Lidbeg P., Martinson K., Boso W. T. K. and Johansson E. D. B.: Radioimmunoassay of norethindrone: peripheral plasma levels after oral administration to humans and rhesus monkeys. *Contraception* **9** (1974) 265–278.
4. Programme for the Provision of Matched Assay Reagents for RIA Hormones: Special Programme of Research in Human Reproduction. WHO, Geneva (1980).
5. Rodriguez G., Faundez-Latham A. and Atkinson L.: An approach to the analysis of menstrual patterns in the critical evaluation of contraceptives. *Stud. Fam Plann.* **7** (1976) 42–51.
6. Shain R. and Potts M.: Need for and acceptability of long-acting steroidal contraception. In *Long-acting Contraceptive Delivery Systems*. Lippincott, Harper & Row, Philadelphia (1983).
7. Toppozada H. K.: Norethisterone enanthate injectable contraceptive. *Long-acting Contraception* (1983) pp. 104–108.
8. Weiner E. and Johansson E. D. B.: Plasma levels of norethindrone after i.m. injection of 200 mg norethindrone enanthate. *Contraception* **11** (1975) 419–425.
9. WHO Task Force on Long-Acting Agents for the Regulation of Fertility: Multinational comparative clinical trial of long-acting injectable contraceptives: norethindrone enanthate in two dosages regimens and depot medroxyprogesterone acetate. A preliminary report. *Contraception* **25** (1982) 1–11.