Upsala J Med Sci 89: 179–188, 1984

Pharmacodynamics of a Contraceptive Vaginal Ring Releasing Norethindrone and Estradiol: Ovarian Function, Bleeding Control and Lipoprotein Patterns

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ABSTRACT

A new contraceptive vaginal ring (CVR), releasing ~700 μg of norethindrone (NET) and ~140 μg of estradiol (E2) daily, was studied in eleven women for a total of 61 21-day cycles. Ovarian function, as judged by plasma progesterone (P) and E2 levels, and plasma NET levels were studied by weekly blood samples in 30 cycles. The lipoprotein pattern was studied before, after two and six months of treatment and one month after completed treatment.

The CVR gave rise to stable plasma NET levels which however varied considerably between individuals. Signs of luteal activity/ovulation were encountered in 4/30 cycles, all in subjects with the lowest NET plasma levels. E2 levels above 250 pmol/l, indicating follicular activity, were encountered in 22/30 cycles. Breakthrough bleeding and spotting appeared in 40/61 cycles and in 12 per cent of the treatment days. Bleeding control was significantly better in the same subjects when using a CVR releasing levo-Norgestrel and E2.

Serum and HDL cholesterol concentrations decreased significantly by 10-12 per cent during treatment. The ratios between apolipoproteins A-I and A-II on one hand and HDL cholesterol on the other increased significantly and the ratio apolipoprotein A-I:A-II decreased significantly, indicating a change in the lipoprotein composition. These changes are qualitatively similar but quantitatively not as pronounced as with the more extensively studied 1-Ng/E2 CVR. The difference in clinical performance and in the effects on the lipoprotein pattern between the presently studied CVR and the 1-Ng/E2 CVR is most likely the result of not using equipotent doses of gestagen in the CVRs.

INTRODUCTION

Clinical trials of contraceptive vaginal rings (CVR) made of Silastic® releasing levo-Norgestrel (l-Ng) and estradiol (E2) have shown that they have a good contraceptive effect and a high acceptability (1, 2). The use of l-Ng/E2 CVRs is associated with a decrease in high density lipoproteins (HDL) (3,4,5) which has caused some concern, because low such values are statistically associated with a high incidence of ischaemic heart disease in prospective studies (6,7). In a pilot project we investigated the clinical effects of CVRs releasing alternative gestagens and also studied the effects on the lipoprotein pattern (8). Although the effects on the lipoprotein pattern were much less pronounced with rings releasing norethisterone (NET), medroxyprogesterone acetate, megestrol acetate and progesterone (P), these rings did not seem acceptable from the clinical point of view because of insufficient effect on ovarian function and poor bleeding control. The present study was made to further evaluate a CVR releasing NET and E2.

MATERIAL AND METHODS

Subjects: Twelve women who had previously participated in a clinical trial of a CVR releasing l-Ng and E2 (1,2) and completed at least one year of treatment were enrolled in this study. Enrolment of women with previous experience of another CVR was made to facilitate a comparison of bleeding patterns. One subject dropped out of the study before treatment was initiated because of a prolonged amenorrhoea. All other subjects had regular menstrual cycles. Some relevant data on the subjects are shown in Table 1. All subjects were on normal diet and none used any form of other continuous medication or excessive amounts of alcohol.

Table 1. Entry characteristics of subjects participating in the study.

Subj. no	Age (years)	Weight (kg)	Height (cm)	Parity (no)	Physical activity*	Smoking (cig./day)
1	24	57.5	159	0	1	<10
2	26	63.3	160	0	3	10-15
3	31	60.0	175	2	3	20
4	21	60.0	170	0	2	0
5	36	58.5	166	3	2	10-15
6	29	54.0	168	0	3	0
8	25	63.2	167	0	3	10
9	29	50.0	158	0	3	10
10	26	62.5	165	2	2	0
11	29	68.5	164	2	2	15-20
12	23	57.5	163	0	2	15
Mean	27	59.5	165	* 1=low, 2=ordinary,		
range	21-36	50.0-68.5	158-175	3=regularly exercising		

Contraceptive vaginal ring: The rings used were of the "shell" design (9) and were impregnated with NET and E2. The in-vivo release rate of the two steroids was calculated by determination of the amount of steroid left in the ring after use.

Design of the trial: After a control cycle, during which no steroid use was allowed, the CVR was inserted into the vagina by the woman on the fifth day of the menstrual cycle. The CVR was left in the vagina for 21-day periods with a seven day interval between each period. Each woman used a CVR for six 21-day periods, changing to a new CVR after three periods. Treatment was followed by a recovery cycle without steroid treatment. All subjects kept a bleeding record which was compared to the bleeding record kept during the previous trial of the l-Ng/E2 CVR.

Blood sampling: Venous blood samples for the determination of P, E2 and NET were collected weekly during treatment cycles one, three and six. Fasting venous blood samples for lipoprotein determinations were collected immediately before treatment was initiated, at the end of treatment cycles two and six and at the return of menstruation in the recovery cycle.

Steroid determinations: Plasma levels of P (10), E2 (11) and NET (12) were determined by radioimmunoassay.

Analysis of serum lipids, HDL lipids and lipoproteins: The serum triglyceride and cholesterol concentrations were determined in an isopropanol extract by semiautomatic methods in a Technicon Auto Analyzer II (13) in whole serum and in the supernatant, after precipitation of the very low and low density lipoproteins using a heparin-manganese chloride solution (14).The concentrations of apo B, A-I and A-II in whole serum (15) were determined by an immuno electrophoresis technique using the concentration in a pooled serum as reference, which was given the value of 100%. The concentrations are thus expressed in arbitrary units (AU).

Statistics: Means and standard deviations were calculated by ordinary methods. A linear model including factors for subjects and timepoint was used, and hypotheses of equal timepoint means were tested by contrasts in the timepoint factor. Hypotheses of equal proportions were tested by the usual normal approximation or by a χ^2-test.

RESULTS

Ten subjects completed six treatment cycles each. One subject (no. 12) withdrew from the study after one cycle because of breakthrough bleeding and acne. Subject no. 9 completed six cycles of treatment but was unable to give blood samples after her third cycle as she had left the area. The results of these two patients have been included in the statistical analysis where appropriate.

Bleeding control: Table II shows the number of days with bleeding or spotting (S) during the trial and the number of days with breakthrough bleeding (BTB) and S during CVR use. As can be seen the subjects had bleeding or S in 24 per cent of total treatment period and BTB/S in 12 per cent of the treatment days. Both figures are significantly higher than during the corresponding period of l-Ng/E2 CVR use. The difference is more marked for BTB/S. BTB/S occurred in 40/61 cycles with the NET/E2 CVR and in 26/61 cycles with the l-Ng/E2 CVR.

Table 2. Bleeding control. A comparison between the NET/E2 and the l-Ng/E2 CVR in the same subjects.

CVR type	Total number of bleeding days[1]	Days with BTB/S[2]
NET/E2	408 (24%) *	158 (12%) ***
l-Ng/E2	359 (21%)	66 (5%)

[1]Calculated on 61 28-day cycles
[2]Calculated on 61 21-day periods
*:$p<0.05$, ***:$p<0.001$ - χ^2-test

Steroid release: The mean NET release from the CVRs was 692 µg/day (SD=83, range 540-865 µg/day). The mean E2 release was 138 µg/day (SD=23, range 111-175 µg/day).

NET plasma levels: A total of 30 treatment cycles were followed with weekly NET determinations. As shown in Table 3, the overall mean values for cycles one, three and six did not differ significantly. The individual subjects differed in their mean NET levels, as can be seen from the range (4.8-14.7 nmol/l), but each individual remained on a stable level in all three cycles studied, with a mean difference between the highest and lowest value in each subject of 1.6 nmol/l and the largest intraindividual difference being 2.7 nmol/l.

Table 3. Plasma NET levels (nmol/l) during treatment.

	Cycle 1	Cycle 3	Cycle 6
Mean	7.9	8.0	8.3
SD	2.26	2.85	1.57
range	4.8-13.3	4.8-14.7	6.3-10.8
n	11	10	9

Ovarian function: Progesterone values indicating ovulation/luteal activity were encountered in four out of 30 cycles studied. In three of these the P values were above 20 nmol/l and in one just above 5 nmol/l. Three of the cycles with luteal activity were found in one subject. The two subjects showing luteal activity also had the lowest mean plasma levels of NET. Signs of follicular activity, arbitrarily defined as E2 levels above 250 pmol/l, were encountered in 22 of 30 cycles. In nine of these cycles E2 levels were above 500 pmol/l.

Lipoprotein metabolism: Serum cholesterol and HDL cholesterol were significantly decreased during treatment by 10-12 per cent when compared to pre- or post-treatment values (Table 4). There was no significant further decrease from the second treatment cycle to the sixth. The pre- and post-treatment values did not differ in any of the variables studied. Serum and HDL triglycerides did not change significantly during treatment.

Table 4. Concentrations (mean ±SD) of triglycerides (TG)(mmol/l) and cholesterol (CHOL)(mmol/l) in serum and in HDL before, at the end of cycles 2 and 6 and at the end of the recovery cycle after treatment.

	Serum		HDL	
	TG	CHOL	TG	CHOL
	mean SD	mean SD	mean SD	mean SD
Pre-treatment	1.32±0.57	4.85±1.19	0.30±0.05	1.30±0.31
Cycle 2	1.10±0.44	** ††† 4.35±0.90	0.27±0.06	† 1.20±.21
Cycle 6	1.05±0.28	* †† 4.31±0.99	0.26±0.04	* †† 1.14±0.25
After treatment	1.28±0.53	4.77±0.86	0.25±0.04	1.37±0.31

* significant difference from pre-treatment values
† significant difference from post-treatment values
* or †; $p<0.05$, ** or ††; $p<0.01$, *** or †††; $p<0.001$

The apo B, A-I and A-II concentrations were not significantly affected by the NET/E2 CVRs (Table 5). However, the ratio between the A-I concentration and the HDL cholesterol concentration and that between A-II and HDL cholesterol were significantly increased during treatment (Table 5). Furthermore, the ratio between apo A-I and A-II was significantly decreased during treatment (Table 5).

Table 5. The concentrations (mean ±SD) apolipoprotein (apo) B, A-I and A-II (arbitrary units=AU) and the ratios apo A-I:HDL cholesterol, A-II: HDL cholesterol and A-I:A-II before, at the end of the second and sixth treatment cycles and at the end of the recovery cycle after treatment.

	Apo B	Apo A-I	Apo A-II
Pre-treatment	106 ± 34	104 ± 11	104 ± 16
Cycle 2	102 ± 23	100 ± 15	105 ± 18
Cycle 6	107 ± 29	96 ± 12	102 ± 14
After treatment	104 ± 30	101 ± 15	101 ± 17
	A-I/ HDL-chol		A-II/ HDL-chol
Pre-treatment	82 ± 14	83 ± 19	1.01 ± 0.10
Cycle 2	† 85 ± 13	†† 90 ± 20	* † 0.96 ± 0.09
Cycle 6	* †† 87 ± 15	* ††† 94 ± 22	** †† 0.94 ± 0.08
After treatment	75 ± 11	76 ± 17	1.01 ± 0.11

*Significant difference from pre-treatment values
†Significant difference from post-treatment values
* or †; $p<0.05$, ** or ††; $p<0.01$, *** or †††; $p<0.001$

DISCUSSION

Treatment with the NET/E2 CVR used in this study results in even plasma levels of NET throughout a six month period of treatment. The mean levels found are about four times higher than those found during treatment with a CVR releasing 200 µg and ten times higher than those with a CVR releasing 50 µg of NET (16). The interindividual differences in plasma levels do not seem to be due to differences in release rates of the CVRs. More likely they are the result of interindividual differences in NET metabolism. In a pharmacokinetic study of NET after oral administration, Odlind et al. (17) found a

variation of the NET half-life 8-24 hours after drug administration of 4.9-15.1 hours, which would support this interpretation of the present data.

The NET levels found are considerably higher than those found in women using subcutaneous rods releasing NET (18) where ovulation is suppressed at NET levels of about 3 nmol/l. In this study, ovulations were encountered at plasma levels of about 5 nmol/l. With CVRs releasing 200 µg of NET used continuously and giving plasma levels of 1.8-3.0 nmol/l, luteal activity was observed in 26 per cent of the cycles (16). This apparent discrepancy between NET levels and ovarian activity is probably explained by the difference in treatment schedules. The one week interval between treatment cycles used in this study allows unopposed follicular development, whereas with the continuous mode of treatment ovarian activity is constantly counteracted.

With the more extensively studied 1-Ng/E2 CVR, ovarian activity seems to be almost completely inhibited (19, 20), also when used in a scheme with one week intervals between the treatment cycles. Considering the frequency of elevated E2 levels encountered in this study, it must be concluded that the two rings are not equipotent. This difference in potency is also reflected in the difference in effect on lipoprotein patterns and in the bleeding control.

The effects on the lipoprotein pattern of the presently used CVR is qualitatively similar but quantitatively not as pronounced as with the 1-Ng/E2 CVR (3, 4, 5). Both serum and HDL cholesterol decreased by about 10-12 per cent with the NET/E2 CVR whereas the decrease with the 1-Ng/E2 CVR is 15-25 per cent. Treatment with the NET/E2 CVR also seems to be associated with a change in composition of the lipoproteins as indicated by the increase in protein:lipid ratio which has also been demonstrated for the 1-Ng/E2 CVR (4). The ratio A-I/AII is higher in the subfraction of HDL designated HDL_2 than in HDL_3. The significant change in the A-I/A-II ratio is interpreted as indicating a decrease of HDL_2 relative to HDL_3. This interpretation is in agreement with findings of Roy et al. (3) in a study of the 1-Ng/E2 CVR where a more pronounced effect on the HDL_2 fraction than on the HDL_3 fraction was demonstrated. The HDL_2 is the subclass that seems to be related to the development of atherosclerosis (21). Therefore, the rather pronounced decrease of HDL during 1-Ng/E2 CVR treatment has caused some concern when initiating investigations of alternative gestagens for their metabolic effects when administered from a CVR. However, it is accompanied by a decrease of the LDL cholesterol, which seemed to occur also in the present study, where the serum cholesterol decrease was larger than the HDL cholesterol decrease. The present results indicate that the NET effects are similar to those of 1-Ng

on lipoprotein metabolism. However, a definite quantitative comparison with regard to lipoprotein effects is not possible due to the differences in the endocrine profile of the two types of CVR.

In untreated menstrual cycles, bleeding occurs in about 18 per cent of the days (16). In the present study, bleeding occurred in 24 percent, and in the same subject when using the 1-Ng/E2 CVR in 21 per cent of the days. This relatively small increase in the number of days with bleeding would probably not be a problem if the days of bleeding were predictable. However, with the NET/E2 CVR 39 per cent of the bleeding days were days of treatment when bleeding is not expected by the woman. With the 1-Ng/E2 CVR the corresponding figure in the same women was only 18 per cent. Unpredictable bleeding is a major drawback for any new contraceptive method.

In a comparative study of a minipill containing 300 μg of NET and two CVRs releasing 50 or 200 μg of NET and used continuously, Landgren et al. (16) found that bleeding occurred in 22, 24 and 35 per cent of treatment days respectively. Signs of luteal activity and normal ovulatory-like patterns were observed in 64, 93 and 26 per cent of the cycles.The presently used CVR exerts a bleeding control that seems to be at least comparable to these three methods and a clearly better ovulatory inhibition (4/30 cycles:13 per cent with luteal activity). The minipill is a widely distributed, used and accepted method of contraception.

The results of this study seem to show that the NET/E2 CVR used in this study would not be as acceptable as the 1-Ng/E2 CVR which has been found safe and acceptable in extensive clinical trials. An increase in the NET release from the CVR would probably, judging from the experience with CVRs releasing 1-Ng, improve bleeding control but would also give rise to changes in the lipoprotein patterns of the same magnitude as the 1-Ng/E2 CVR.

In conclusion, the poor bleeding control and the insufficient effects on ovarian function indicates that the NET/E2 CVR used in this study is not as acceptable as the 1-Ng/E2 CVR when compared in subjects who earlier were positive responders to that type of CVR.

ACKNOWLEDGEMENTS

This work was undertaken as part of the contraceptive development program sponsored and coordinated by the International Committee for Contraception Research of the Population Council, New York, New York.

The financial support provided by the International Development Research Centre of Canada, the U.S. Agency for International Development (Grant AID/pha-G-1116), the Ford Foundation, the Rockefeller Foundation and the Geo. J. Hecht Fund is gratefully acknowledged. The content does not necessarily reflect the policy of any of the funding sources. The study was also supported by grants from the Swedish Medical Research Council (3495, 5446, 5646).

We are indebted to Dr Theodore M. Jackanicz of the Population Council, New York, N.Y., U.S.A. for manufacturing the CVRs and determination of release data.

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