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Estradiol delivery by vaginal rings: potential for hormone replacement therapy[1]

Harold A. Nash[a,*], Vivian Brache[b], Francisco Alvarez-Sanchez[b], Theodore M. Jackanicz[a], Troy M. Harmon[c]

[a]*The Population Council, 1230 York Avenue, New York, NY 10021 USA*
[b]*PROFAMILIA, Santo Domingo, Dominican Republic*
[c]*FEI Technologies Plainsboro, NJ, USA*

Abstract

Objectives: To determine if delivery of estradiol from elastomeric vaginal rings gives estradiol blood levels in the range associated with effective estrogen replacement therapy and to determine the relation between in vitro estradiol release from the rings and blood levels in vivo. Secondary objectives related to changes in lipoprotein cholesterol, changes in climacteric symptoms, and evaluation of acceptability to users. *Methods*: Three ring variants releasing approximately 100, 150 and 200 μg/day of estradiol in vitro were used through 22 days in 21 postmenopausal women, 7 on each dose level. Blood samples for measurement of estradiol were taken at 3–4 day intervals. Lipoprotein cholesterol was measured before and at the end of treatment. Women were questioned about climacteric symptoms and about their satisfaction with the ring. *Results*: Mean serum estradiol levels for the three groups of rings were 63 ± 6, 94 ± 5 and 136 ± 13 pg/ml for the 100, 150 and 200 μg/day rings, respectively. FSH levels declined during ring use and the maturation values of cells collected on vaginal swabs markedly increased. Total and LDL cholesterol were significantly reduced and HDL cholesterol was not significantly changed. All women reported relief of postmenopausal symptoms. Vaginal discomfort during the first 3 days of use was reported by 12 women but overall satisfaction with the method was high. *Conclusions*: Women using the vaginal rings attained estradiol blood levels compatible with control of climacteric symptoms and bone loss. The relation between in vitro estradiol release and blood levels in vivo was essentially identical for all 3 doses. The use of vaginal rings to deliver estradiol for hormone replacement therapy is judged to merit further evaluation. Copyright © 1997 Elsevier Science Ireland Ltd.

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1. Introduction

The administration of exogenous estrogen to postmenopausal or ovariectomized women is a

* Corresponding author. Tel: 212 3278692 (business)/Tel: 201 7685943 (home); fax: 212 3277678.

[1] Study Site: Biomedical Research Department, PROFAMILIA, Santo Domingo, The Dominican Republic.

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well-recognized and growing practice. It demonstrably relieves such consequences of estrogen deficiencies as hot flushes, atrophic vaginitis, and senile urethritis and there is much evidence of reduction in the incidence of coronary heart disease and bone fragility [1].

Several modes of estrogen administration are in use. They include daily oral administration of conjugated equine estrogens or micronized estradiol and transdermal patches changed every 3–5 days. In Europe, gels applied daily to the skin and implants or pellets placed subdermally are also used. Other products releasing very low doses of estrogen locally to treat atrophic vaginitis are also available. They include creams, pessaries, vaginal tablets and recently, in Europe, a low dose vaginal ring [2].

The use of a vaginal ring to deliver estrogens has several attractive features. They include the fact that women need give attention to the ring only at the beginning and end of a treatment period or at the end of the effective life of the ring, the avoidance of the skin irritations sometimes associated with transdermal patches, the fact that nothing is visible, and the fact that, as opposed to subdermal delivery, the method is under the woman's control. These advantages are best realized if the ring also delivers enough progestin to prevent endometrial hyperplasia.

This paper reports on the first in vivo studies of a ring which in vitro has shown itself capable of delivering a nearly constant dose of estradiol-17β for several months and which can be manufactured to deliver selected doses over a broad range. The study was designed to ascertain whether the doses of estradiol delivered by the rings gave blood levels within the range generally considered optimum for relief of vasomotor symptoms associated with menopause and at establishing correlation between in vitro delivery and estradiol blood levels.

A secondary objective was to assess the acceptability of this vaginal ring among menopausal women, and to record relevant clinical findings in this preliminary short pharmacokinetic study.

2. Materials and methods

2.1. Vaginal rings

Rings were manufactured by Akcess Medical Products Inc. of King of Prussia, PA (since reorganized with the relevant unit being named FEI Technologies). They were of approximately 56 mm overall diameter and 8 mm cross-sectional diameter. They consisted of a core containing 4% estradiol-17β in a matrix of dimethylsiloxane/vinylmethylsiloxane elastomer. The core was coated by a dipping process with a similar silicone elastomer using coating thicknesses of 0.21, 0.28 and 0.47 mm to achieve three different release rates. These rings delivered approximately 200, 150 and 100 μg/day, respectively, in vitro and are identified in this paper by their nominal in vitro release rates.

2.2. In vitro release rate

For measurement of estradiol release in vitro, rings were individually suspended in 250 ml of 1:750 benzalkonium chloride solution contained in 500-ml wide mouth plastic bottles. The bottles were placed on a shaking water bath maintained at 37°C and making 100 one-inch excursions per min. The solutions were changed daily Monday through Friday and the solutions collected on Wednesday, Thursday, and Friday assayed for estradiol by HPLC.

2.3. In vivo study

After protocol approval by the Population Council and local Investigational Review Boards and after individual subjects gave informed consent, women were enrolled at PROFAMILIA in Santo Domingo, The Dominican Republic. Seven women were enrolled on each of the three dosage variants. The rings were used for 22 days with blood samples being taken for assay at days 1, 3 or 4, 7/8, 10/11, 14/15, 16/17, 19/20, 21/22 and 2 days post-treatment. Blood samples were also taken 1 week pretreatment and immediately before ring insertion.

Women were required to be 3–15 years postmenopausal, in general good health, to have experienced at least 6 months of secondary amenorrhea and to have a plasma FSH level above 40 mIU/ml. They had not used estrogen therapy in the 10 days prior to pretreatment blood sampling. Contraindications were cancer, undiagnosed vaginal bleeding, hypertension, vaginal abnormalities or vaginal disorders, and thromboembolic disorders.

All women underwent a gynecological exam prior to admission and at the end of the 3 week treatment period. A bleeding record was kept during the study and continued until 1 month after discontinuation.

Fasting blood samples for the measurement of cholesterol, HDL-cholesterol and LDL-cholesterol were also taken at admission and on the day of the removal of the ring.

At the end of treatment, women responded to a simple questionnaire, regarding acceptability of the rings and side effects.

2.4. Hormone assay

Estradiol was measured in serum by a solid-phase ^{125}I radioimmunoassay (Diagnostic Products Corp.). Interassay and intraassay coefficients of variation were 9.2 and 5.3%, respectively. FSH was assayed by liquid phase second antibody ^{125}I radioimmunoassay provided by the World Health Organization reagent program. Inter- and intraassay coefficients of variation were 13.7 and 5.6%, respectively. The laboratory participates in the WHO External Quality Assessment Scheme for Reproductive Hormones.

2.5. Vaginal cytology

Cells collected from vaginal swabs were examined microscopically and observations expressed by the maturation value of Meisels [3]. The percentage of cells of each type is multiplied by value factors: superficial cells = 1.0, intermediate = 0.5 and parabasal = 0. The sum of these products is the maturation value.

Table 1
Characteristics of women at entry into the estradiol ring study

	Ring series		
	100	150	200
N	7	7	7
Age ± S.D.	53 ± 4	54 ± 3	54 ± 5
Weight, kg ± S.D.	64 ± 10	64 ± 16	65 ± 12
E_2^a pg/ml ± S.D.	7.0 ± 3.1	8.0 ± 4.2	9.9 ± 8.2
FSH mIU/ml ± S.D.	51 ± 24	83 ± 57	65 ± 34

[a]Estradiol.

3. Results

3.1. Demographic

Characteristics at entry into the study of the women using the three ring variants are summarized in Table 1.

3.2. In vitro release

In vitro release rates were determined on new rings and on rings returned after in vivo use through 22 days. The findings are summarized in Table 2. They show the in vitro release to match well the design goals and indicate no loss of release capacity after the 3 weeks of use in vivo.

Table 2
In vitro estradiol release from new and used rings

	Ring series		
	100	150	200
New rings ($N = 3$)			
μg/day ± S.D.[a]	95 ± 2	154 ± 4	201 ± 4
Mean S.D. among rings[b]	±2	±3	±9
Used rings ($N = 4$)			
μg/day ± S.D.[a]	96 ± 2	149 ± 5	203 ± 6
Mean S.D. among rings[b]	±4	±4	±6

[a]S.D. based on day-to-day variation for group over a 10-day period with measurements on days 2, 3, 4, 8, 9 and 10.
[b]Mean S.D. among rings over the same measurement period as for the new rings.

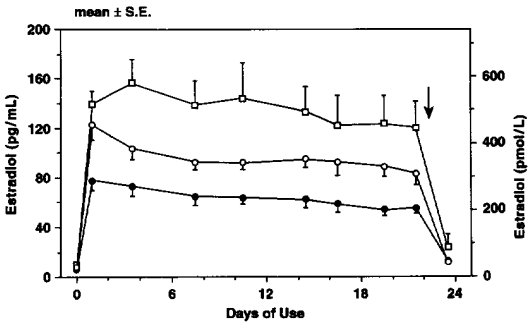

Fig. 1. Serum estradiol levels before, during, and 2 days after use of rings delivering 200 (□), 150 (○) or 100 (●) μg/day of estradiol. The arrow indicates ring removal. $N = 7$ each group.

3.3. Estradiol blood levels

Blood levels of estradiol among users of the three ring variants are shown in Fig. 1. Mean levels were 63 ± 6, 94 ± 5 and 136 ± 13 pg/ml (231, 345 and 500 pmol/l) for the three ring types. The blood levels reflect almost perfectly the differences expected among ring types based on in vitro measurements. The relation between in vitro release rates for the three ring variants are represented by the ratios 1:1.6:2.1; those for blood levels by the ratios 1:1.5:2.2. Correlations with in vitro release are further delineated in Table 3. They show that 1 μg/day estradiol release in vitro corresponds to a blood level of about 0.64 pg/ml (2.4 pmol/l) in vivo. Subtracting the pretreatment estradiol levels to estimate the increment in serum levels corresponding to an increment of 1 μg/day in vitro release, yields a mean of 0.58 pg/ml (2.1 pmol/l). Differences in serum estradiol concentrations among individual women in each group were large at the extremes, with the differences between the lowest and highest as represented in Table 3 being 2-fold for the 100 μg group and 3.7-fold for the 200 μg group.

3.4. FSH responses

As expected, FSH concentrations were decreased during ring use (Table 4). Although there is a hint of greater response as a function of dose, final FSH concentrations do not differ significantly among the groups.

3.5. Vaginal response

Vaginal cytology was evaluated pretreatment and at the end of treatment. The findings in terms of maturation value are represented graphically in Fig. 2. It is seen that all subjects have responded with a shift toward an estrogen-influenced vaginal endothelium.

3.6. Lipoprotein cholesterol

A significant reduction in total cholesterol and LDL-cholesterol was observed. The mean reduction was 6% and 12%, respectively ($P < 0.01$). The mean baseline levels of cholesterol and LDL-cholesterol, were 192.4 ± 41.4 (S.D.) and 131.5 ± 39.0 mg/dl, dropping to 181.0 ± 32.4 and 114.4 ± 38.8 mg/dl, respectively. HDL-cholesterol levels had an insignificant increase (38.2 ± 9.8 to 40.3 ± 9.9 mg/dl).

3.7. Clinical observations

Several women reported spontaneous complaints during the course of the study: 3 women reported mastalgia (none with the low dose, 2 with the intermediate dose and 1 with the high dose). Lower abdominal pain was also reported in 3 of the subjects. Only one subject had a spontaneous expulsion of the ring. She reinserted it immediately.

Withdrawal bleeding occurred in 2 of the 7 subjects on the high dose, and none among women in the remaining dose groups. This bleeding ensued within the first 6 days post-removal. Three additional subjects (one in each dose) reported 1 day spotting 3–4 days after removal of the ring.

When questioned regarding their general experience with the ring, all 21 women replied it had been good ($n = 17$) or very good ($n = 4$). When specifically asked, 12 women reported some vaginal discomfort initially, mostly during the first to third day. Of the 21 women enrolled, 19 reported having menopausal symptoms prior to enrolment; mostly hot flushes ($n = 15$), nervousness, and irritability. At the end of the 3-week period of use, all 19 women reported relief of these symptoms. Re-

Table 3
Correlation between in vitro release rates and serum levels

Ring series	Serum estradiol pg/ml			Ratio: pg/ml/μg/ring/day
	In vitro release μg/ring/day[a,b]	Mean ± S.D.[c,d]	Range[c]	
100	98 ± 5	63 ± 6 (231)[e]	43–84	0.65
150	158 ± 5	94 ± 5 (345)	72–127	0.60
200	203 ± 5	136 ± 13 (500)	61–227	0.67

[a]Mean of days 4 to 20.
[b]S.D. reflects variations in the mean for the group from one determination to another.
[c]Mean of days 3/4 through 19/20. This interval was selected to correspond to the interval for which in vitro results are available. Including days 1 and 21/22 has little effect on the values.
[d]S.D. reflects variation among women.
[e]Mean value in pmol/l indicated in parenthesis.

garding the appearance of the ring, 3 women found it was somewhat large, whereas the remaining 18 found the size acceptable.

4. Discussion

The goal of determining the correlation between in vivo release rates and serum estradiol levels has been satisfactorily attained. The ratio has been essentially constant for all three dosing levels with 1 μg per day of in vitro release equalling 0.64 pg/ml in serum. Although the differences in blood levels of estradiol were relatively large among the women in each group, the small differences in in vitro release rates among returned rings (Table 2) indicates that the differences among women in estradiol levels must result from differences in metabolism or absorption.

Over the period of 22 days of ring use, there was a decline in serum estradiol concentrations of 31, 27 and 21% based on linear regressions for the 100, 150 and 200 μg groups, respectively. In vitro release rates of recovered rings indicate this decline was not due to loss of release capacity (Table 2). That it is probably due to changes in the absorptive capacity of the vaginal epithelium is indicated by the work of Pschera, Hjerpe and Carlstrm [4]. They found large and statistically significant differences in estradiol, estrone and progesterone serum concentrations between women with atrophic vaginal mucosa and moderately proliferated vaginal mucosa when suppositories containing estradiol-17β and progesterone were applied vaginally. The direct release of estradiol into the vagina might be expected to produce a highly estrogenized vaginal epithelium and this was confirmed by cytology assessments (Fig. 2). Similar decreases in circulating estradiol concentrations in a first cycle of use of an estradiol ring were recorded in a publication by Englund, Victor and Johansson [5]. Data on two subjects in that

Table 4
Effects of ring use on FSH concentrations

Ring series	Mean FSH in mIU/ml		
	Before	At end of use	Percent decrease
100	51 ± 24	37 ± 22	27
150	83 ± 57	34 ± 24	59
200	65 ± 34	24 ± 21	63

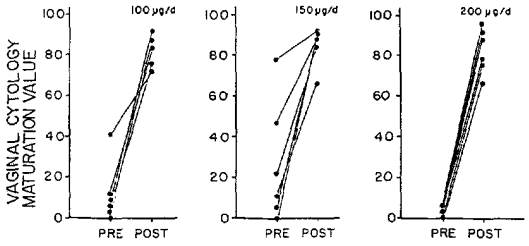

Fig. 2. Vaginal cytology maturation changes during estradiol ring use by postmenopausal women.

study who extended use beyond one cycle indicate levels in the second cycle similar to those at the end of the first cycle.

Based on reported experiences with transdermal and implant estradiol delivery, the appropriate delivery rate is judged to lie in the lower half of the range covered by rings included in the trial. Comparison with transdermal and implant delivery is considered the appropriate standard since conversion to estrone is much more prominent when administration is by the oral route.

Estradiol blood levels from transdermal patches nominally delivering 50 μg/day ranged in several studies from means of 33 to 80 pg/ml [6–12] and those from 100 μg patches from 65 to 105 pg/ml [8,11–16]. Significant relief of hot flushes has been reported for 50 μg/day patches and a 50 μg/day transdermal cream [6,8–10]. Improvement in a variety of quality of life measures has been reported for 50 μg/day transdermal patches [9].

Maintenance and enhancement of bone density has been reported among postmenopausal women using 50 and 100 μg/day transdermal patches [11,17–19]. In studies with estradiol implants, increased bone density was correlated with estradiol levels over a broad range [20]. The highest levels gave the greatest increase in bone density but the change in increment from estradiol blood levels of 200 pmol/l (54 pg/ml) to 800 pmol/l (218 pg/ml) was relatively modest. The authors note the high variability among women and advise that in the absence of bone density monitoring the goal should be estradiol levels of at least 300 pmol/l (82 pg/ml). The intermediate dose ring used in the present study attains levels in this range.

Although the major objective of this study was to determine if the vaginal rings were capable of delivering adequate estradiol levels and to correlate these levels with the in vitro release rate, we can also conclude that the ring was highly acceptable to the participating women, and that improvement of menopausal symptoms and of lipid profiles was achieved with all three ring doses. Regarding the initial vaginal discomfort reported by approximately half of the women, we suspect that the gynecological exam (introduction of the vaginal speculum in an atrophic vagina) may have contributed. The possibility may exist, however, that the ring itself might have been tight in the atrophic vagina. In any case, this discomfort disappeared completely after the first few days of use. With such few subjects and such a short-lasting study, we were not able to assess whether differences in clinical performance existed between the three doses.

In further development of the estradiol ring, studies aimed at confirming its effectiveness after 6 months are underway.

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