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"Pharmacokinetic and Pharmacodynamics of Three Doses of Oestriol in Healthy Postmenopausal Women Following Continuos Vaginal Ring Delivery for 21 Days"

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ABSTRACT:

In healthy postmenopausal women receiving treatment with a vaginal ring at continuous delivery rates of 0.125 (Test 1), 0.250 (Test 2), or 0.500 mg day–1 (Test 3) for 21 days, the AIMSE evaluated the oestriol pharmacokinetics, pharmacodynamics, and safety. METHODS One application of Tests 1, 2, or 3 was given to each of the thirty-one individuals. The technique of liquid chromatography coupled tandem mass spectrometry was used to ascertain the oestriol plasma concentration. The pharmacodynamics were evaluated by measuring serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and sex hormone-binding globulin, as well as maturation value (MV) and vaginal pH. To assess safety, adverse events, endometrial thickness, and local tolerability were considered. RESULTS: For the area under the plasma concentration (AUC) curve up to the most recent measurement, the 90% confidence interval of the coefficient/slopeβwas 0.5997–1.174%, for the AUC extrapolated to infinity, it was 0.5838–1.115%, and for the maximum plasma concentration, it was 0.2408–0.943%. The dose proportionality was unavoidable for maximum plasma concentration, although a statistically significant departure from proportionality was seen for AUC. Higher delivery rates were associated with a more noticeable decline in the FSH and LH curves; however, sex hormone-binding globulin did not exhibit this behaviour. For each formulation, the treatment's impact on vaginal pH and MV was comparable. Every product had a 70–80% rise in MV, and there was a shift in the distribution of parabasal, intermediate, and superficial cells in Favor of superficial cells. During treatment, the vaginal pH readings significantly dropped. Endometrial thickness was not affected in a dose-dependent manner. FINAL VERDICT Each formulation produced the ideal amount of local impact by releasing enough oestriol. On the other hand, there was no variation in the surrogate parameters for clinical efficacy between formulations. For FSH and LH, however, a dose-dependency was well shown. The product was safe and well-tolerated.

1. Introduction

The hormonal shift that occurs after menopause marks the end of the reproductive phase for women. The ovarian oestrogen and progesterone synthesis declines, which results in a drop in the levels of circulating oestrone, oestradiol, and sex hormone-binding globulin (SHBG) and an increase in luteinizing hormone (LH) and follicle-stimulating hormone (FSH)[1-3]. Hormone replacement therapy (HRT), which effectively relieves vasomotor symptoms like hot flashes, intense sweating, and vaginal atrophy, which may affect 50–60% of post-menopausal and bilaterally ovariectomized women, can be used to treat the severe discomfort associated with this normal stage of life. Hot flashes can be less frequent and less severe when Oralor transdermal oestrogen therapy is used [4-6].Europe, South America, and the USA all sell different vaginal rings for hormone replacement therapy (HRT); nevertheless, as of right now, only oestradiol-containing vaginal rings are offered for sale [6, 7].One of the three primary natural estrogens in the metabolism of female hormones is estriol. Aside from oestradiol and oestrone, it is one of the most significant oestrogens in

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terms of quantity [8]. The effects of this medication on The engagement of certain oestrogen receptors in the target tissues mediates the effects of estrogens [8, 9]. In the typical female cycle, the oestriol plasma concentrations are around 7.9 pg ml–1 in days 5–7 and 11.1 pg ml–1 in days 20–22. Estrogen levels in postmenopausal women are 6.0 pg ml–1 [10]. It is eliminated as conjugated and unconjugated 2-hydroxy oestriol following more 2-hydroxylation. Urine contains more than 95% of the metabolized estrogen, mostly in the form of glucuronides [8].Oestriol-containing tablets, suppositories, ovules, gels, and creams are only a few of the items that have been promoted as oral or vaginal solutions. These drugs have demonstrated certain benefits over other oestrogens and are being used for HRT at levels of up to 2 mg day–1 [11]. Since oestriol does not promote endometrial growth and hyperplasia, it has been demonstrated to be safer than estrone and oestradiol[12]. The elimination half-life (t1/2) of oestriol is short because it does not bind to SHBG. Following repeated dosages, oestriol has a decreased oestrogenic potency due to its lower affinity for the oestrogen receptorα, higher dissociation constant, and proliferative effect on the vaginal and urethral epithelium via the oestrogen receptorβ [12–14]. Moreover, oestriol has no effect on the function of coagulation [11].When used in conjunction with oestriol, HRT for vaginal atrophy is both safe and efficient. Oestriol encourages the growth of the vaginal epithelium and aids in the return of the normal microbiota and pH to the vagina [14, 15]. Furthermore, oral doses of 8 mg day-1 oestriol have recently been investigated in clinical studies for the treatment of multiple sclerosis in the USA[16]. A very poor systemic bioavailability was demonstrated by single and multiple-dose vaginal injection of pessaries containing 0.03 mg oestriol over 21 days, confirming a positive safety record. After a single dosage, the mean plasma concentration (Cav) was 5.0 pg ml–1, the maximum plasma concentration (Cmax) was 45.1 pg ml–1, and the area under the plasma concentration curve until 12 hours (AUC0–12h) was 119.8 pg ml–1. Nevertheless, following many dosages, the AUC0–12h, ss, Cmax, ss, and Cavat steadystate (Cav, ss) values were, in order, 52.1 pg h ml–1, 2.2 pg ml–1, and 12.7 pg ml–1. The authors speculate that this phenomenon might be caused by an oestriol trophic effect on the vaginal epithelium, which might result in less oestriol being absorbed systemically [13]. This condition was also noted in postmenopausal women who had received a single or many doses of vaginal gel and cream treatment. Following a single application of 20 and 50 mg g–1 oestriol vaginal gel and 1 mg g–1 oestriol vaginal cream, respectively, the Cmax values were 60.57, 106.40, and 210.06 pg ml–1, and in the steady state (after once daily administration for 21 days), the Cmax,ss values were The dosages of 13.77, 22.80, and 89.95 pg ml–1 shown a comparable ability to reverse vaginal atrophy, while the formulations of oestriol 20 and 50 mg g–1 exhibited an extremely favorable safety profile [14].Given the unavailability of oestriol-containing vaginal rings on the market, their effectiveness in alleviating the symptoms of oestrogen depletion, and their safety record, the current study aimed to assess the pharmacokinetics and pharmacodynamics of oestriol in postmenopausal women in good health. They received therapy for 21 days using a vaginal ring containing oestriol at continuous delivery rates of 0.125, 0.250, or 0.500 mg day–1. The development test products contained 100 mg, 300 mg, and 600 mg of oestriol combined. This study assessed if the formulations could be a viable HRT option to treat local vaginal postmenopausal symptoms and whether they released enough oestriol to achieve the greatest local effect in the participants under investigation. The measurement of oestriol was carried out using liquid chromatography coupled tandem mass spectrometry technique that has been verified. A descriptive analysis of the FSH, LH, and SHBG serum levels, as well as an assessment of the gynecological parameters during the course of treatment with varying oestriol dosages, were used to evaluate the pharmacodynamic effects. Safety and tolerability were characterized by recording adverse events (AEs).

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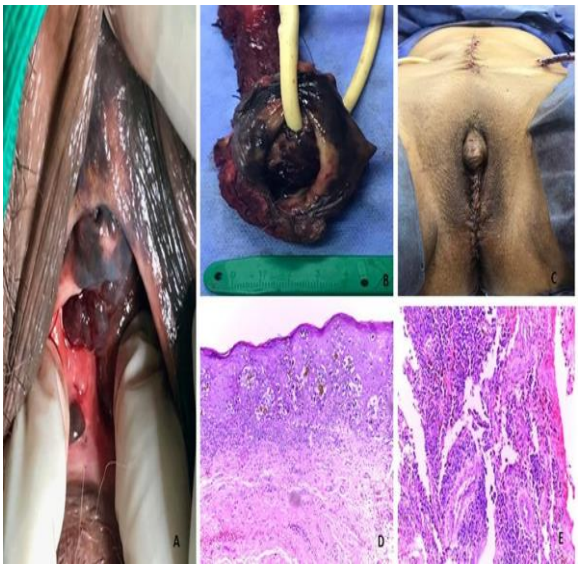

Figure 1 A -Multiple pigmented lesions on the labia majora, vulvar introitus, and vaginal wall, along with a lesion in the region below the urethra. B -Total colpectomy, radical vulvectomy, ureterectomy, and left salpingo-oophorectomy. C -Mitrofanoff reconstruction. D -Proliferation of melanocytes with atypical dispositions on nests and lentiginous pattern in epidermal dermis junction with concentrations of melanocyte ascension. Hematoxylin & eosin x100. E -Malignant melanoma of the vulva with vertical growth diffusely compromising the specimen. Hematoxylin & eosin x100. Note atypical cells of variable size and nuclear morphology with granular chromatin.

f-2 The Stages of Reproductive Aging Workshop +10 staging system for reproductive aging in women

Methods

Topics

This study included thirty-one (31) postmenopausal women in good health The measurement of FSH and oestradiol levels verified the postmenopausal state. Therefore, FSH serum levels were ≥40 mIU l–1 and oestradiol plasma levels were ≤20 pg m–1 for every patient. Additionally, every individual stated that they had not experienced a menstrual cycle for at least a year, indicating that they were post-menopausal. Their kidney, liver, and heart functions were all within normal ranges, as determined by laboratory testing and clinical evaluation (medical history, physical examination, and ECG). Additionally, they tested negative for antibodies to the hepatitis B core antigen, hepatitis C virus, hepatitis B virus surface antigen, and human immunodeficiency.

Based on a polymer matrix formulation idea, the only active medicinal ingredient in the intravaginal rings was estriol. Ethylene vinyl acetate was homogeneously incorporated with oestriol. Using an in vitro technique, the mean release rate of the vaginal rings containing oestriol was measured over a period of 21 days. The Food and Drug Administration recommends using an orbital incubation shaker (130 rpm, 2% sodium lauryl sulfate in purified water at 37 ± 0.05°C) for performing release testing on intravaginal rings. Design and management of the study This phase I trial was conducted in a parallel-group design and was single-center, open-label, randomized (allocation to therapy), balanced, and single-dose. One of the three potential treatments was assigned to the patients at random:T1: One vaginal ring containing 100 mg of oestriol is applied once. written agreement with full knowledge Substances Evestra GmbH, Germany, supplied the test goods (EVE116) for the clinical investigation. Based on a polymer matrix formulation idea, the intravaginal rings contained estriol as the only active medicinal ingredient. In ethylene vinyl acetate, oestriol was uniformly implanted. Using an in vitro system over the course of 21 days, the mean release rate of the oestriol-containing vaginal rings was ascertained. As advised by the Food and Drug Administration for intravaginal rings, the release tests were carried out in an orbital incubating shaker at 130 rpm and 2% sodium lauryl sulfate in purified water at 37 ± 0.05°C.Study methodology and approach This phase I, single-center, open-label, balanced, single-dose, randomized (allocation to treatment) experiment was carried out in a parallel group configuration. Three potential therapies were randomly assigned to the subjects:T1: one vaginally applied vaginal ring containing 100 mg of oestriol T2: One vaginal ring containing 300 mg of oestriol, applied once; T3: One vaginal ring applied once; T2: 300 mg of oestriol, nominally delivered at a rate of 0.500 mg day–1 (Test 2 - EVE116 300/0) over 21 days; T3: One vaginal ring applied once; T3: 600 mg of oestriol, nominally delivered at a rate of 0.500 mg day–1 (Test 3 - EVE116 600/0) over 21 days. During this clinical research, the individuals were admitted to the hospital twice—once for the insertion of the intravaginal rings and again for their removal. Furthermore, after applying the test product, patients were to return to the trial location for ambulatory visits 2, 4, 6, 9, 12, 15, 18, and 20 days later. The subjects placed the intravaginal rings on their own at the location.

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The subjects themselves verified if the intravaginal rings were positioned correctly in the vagina. In also, questioned in both hospital stays and out-of-office visits by research personnel.

Blood testing

Approximately 11 milliliters of blood (containing EDTA as an anticoagulant) were drawn from a vein using a direct venipuncture method or an indwelling cannula with a switch valve to characterize the pharmacokinetic parameters of oestriol in the plasma 0.5 hours before the drug was applied, as well as 1, 2, 4, 6, 12, 24, 48 hours (2 days), 96 hours (4 days), 144 hours (6 days), 216 hours (9 days), 288 hours (12 days), 360 hours (15 days), and 432 hours (18 days) after the drug was applied. Additional blood samples were obtained 6, 12, and 24 hours after the vaginal ring was removed, as well as five minutes before it was removed, or 21 days following application (study day 22).After centrifuging blood samples at 4°C for 2000g×10min, the plasma samples for oestriol measurement were obtained. The samples were then kept at about 70°C until chromatographic analysis. For clarification Blood samples of 8 ml were obtained before and 2, 5, 7, 10,, 13, 16, 19, and 22 days after drug application in order to measure the serum concentrations of FSH, LH, and SHBG. Examination of oestriol in blood The use of liquid chromatography coupled tandem mass spectrometry allowed for the determination of the plasma concentration of oestriol. As an internal standard, oestriol-d3 was employed (IS). Diethyl ether/hexane (70/30, v/v) was used in a liquid-liquid extraction process under yellow-low light conditions to prepare the sample. Using a 150 × 4.6 mm Inertsil ODS-3 C18 column (GL Sciences Inc., Tokyo, Japan) and a model G1311A HPLC system (Agilent, Waldbronn, Germany), oestriol was separated at a flow rate of 1.2 ml min–1 using acetonitrile/water (90/10, v/v) with 0.1% formic acid as the mobile phase. The positive electrospray mass spectrometer API 4000 (MDS SCIEX and AppliedBiosystems, Toronto, Canada) The method employed for oestriol detection was ionization mode (ES+). The multiple reaction monitoring detection mode was used for conducting the analyses.The European Medicines Agency's Guidelines on Bioanalytical Method Validation were followed during the method validation process [17]. The injection of pooled blank humanplasma from various sources (normal, lipaemic, and hemolyzed) has demonstrated specificity and selectivity, and no interference was seen in chromatograms. At the IS and oestriol retention times, there were no conflicting peaks. For values between 0.005 and 0.2 ng ml–1, the calibration curve was linear, and its correlation coefficient was 0.9992. The results were repeatable and reproducible because the inter- and intra batch accuracy values were within 85–115% and the precision of LLOQ and quality controls were less than 15%. Practically speaking, there was no matrix effect, and the overall coefficient of variation for the IS normalized The matrix factors were less than fifteen percent. In short-term (7 h), post-processing (at the autosampler temperature for at least 76 h), three freeze-thaw cycles (from ~20°C to 23°C), and long-term (at least 126 days stored at ~20°C) stability testing, Oestriol and IS did not exhibit any significant degradation. The period of time from the first pharmacokinetic sample taken and the final analytical measurement was considered long-term stability.

Assessments of pharmacodynamics and safety During treatment, cytological MV, vaginal pH, and serum concentrations of FSH, LH, and SHBG were measured as pharmacodynamic measures. The levels of FSH, LH, and SHBG in the serum were measured in a medical diagnostic laboratory (Franceschi, Campinas, Brazil) using a two-phase chemiluminescent (sandwich) immunoassay with magnetic particles and the Immunoassay Access Systems (Beckman Coulter Inc., California, USA). Throughout the whole analytical range, the assay demonstrated inter- and intra-assay imprecision values of less than 10%.The cellular count of the viral smears was used to calculate the MV. The samples were sent to Multipath, Campinas, Brazil, a medical laboratory, for examination and staining using the Papanicolaou technique. Maturation indices (MI) in percentages were computed using the vaginal smear's cytology (parabasal, intermediate, and superficial cells), which were described throughout the screening process, on study day 22 (21 days following ring application), and on study day 42 (21 days following ring removal) at the conclusion of the assessment. The MVs were computed as the product of 1.0×MI of superficial cells and 0.5×MI of intermediate cells. The pH of the vagina was determined by sticking a pH test strip (pH 1-4, Merck®, Darmstadt, Germany) into the vagina's top wall during screening, on study day 22 (21 days after the administration of the ring), and on study day 42 (21 days after the study examination).Endometrial thickness (ET) is a safety-related parameter that was measured using

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transvaginal ultrasonography on study days 15, 21, 20, and 42 (21 days after ring removal) by the sonographer-gynecologist at the imaging diagnostic clinic. Although noreatrix endometrial proliferation was expected under treatment with the planned regimes, ET was measured using this method. Additionally, the documentation of the type and frequency of the test goods was used to evaluate their safety and tolerability. of adverse events in the research sample. Additionally, a gynecologist visually inspected the vaginal mucosa at the screening examination 21 to 2 days prior to the scheduled product administration and at the end of the study examination on day 42 (21 ± 2 days following ring removal).

f-3 Study of Women's Health Across the Nation (SWAN). Percent of cycles without evidence of luteal activity (ELA)

Analyses using statistics

Since this clinical trial was a descriptive pilot study, sample size estimation based on statistics has not been carried out. Every treatment's descriptive statistics were assessed for every pharmacokinetic and pharmacodynamic parameter. AUC0-tlast, AUC0-∞, and Cmax were calculated using the power model as outlined by Gough et al. [18] in order to evaluate dosage proportionality. Terms used to refer to targets and ligands Important protein targets and ligands. The overall analysis set contained thirty-one participants, while the per-protocol set contained thirty (PPS). One participant left the research on day six after withdrawing their consent, while the other due to a day 19 procedure infraction. For the second subject, the data for the pertinent study variables were nearly finished, therefore she was added to the PPS. Data from the participants included in the PPS were used for pharmacokinetic and pharmacodynamic evaluation (n = 10 for each medication). Data from the subjects included in the entire analytic set were used for safety evaluation, demographics and other baseline characteristics Medication The in vitro initial release rate of the oestriol-containing vaginal rings was high and declined markedly until approximately After five days, a plateau phase was attained. Drug concentration determined the elevation of the plateau phase For Test 1, 2, and 3, the mean release rates were 0.165 mg day–1 (ranging from an average of 0.666 mg day–1 on day 1 to 0.086 mg on day 21), 0.318 mg day–1 (ranging from an average of 1.449 mg day–1 on day 1 to 0.164 mg on day 21), and 0.667 mg day–1 (ranging from an average of 3.470 mg day–1 on day 1 to 0.331 mg on day 21). After the in vitro–in vivo correlation was carried out, 0.999872184 was the correlation coefficient.

Pharmacodynamics

serum levels of FSH, LH, and SHBG. Time curves illustrating the mean serum concentration for variations from baseline in FSH, LH, and is SHBG during vaginal application of one vaginal ring of Test 1, Test 2, or Test 3. Every individual exhibited baseline values beyond 20 mIU l–1 for FSH and above 30 mIU l–1 for LH , which are widely recognized as threshold values for the postmenopausal condition. The box plots that while SHBG values gradually increased until day 19, the highest effect for LH and FSH values was obtained on days 2 and 5, respectively, after ring implantation. MV and pH in the vagina. For every therapy, the MV changes were almost the same The modifications began to disappear 21 days following treatment termination. Similar results were seen in the cytology of the vaginal smear (parabasal, intermediate, and superficial cells), which has been described in order to determine the MV The three treatment groups' mean baseline values before the start of the intervention were very comparable, with roughly 50% of the cells being parabasal, 40% being intermediate, and 10% being specialized. Following 21 days (day 22) of therapy, the percentage of superficial cells in the sample was, on average, around 50%. All other cells were classified as intermediate cells; no parabasal cells were found in the samples. Day 42: After 21 days in a row without therapy, superficial cells made up approximately 15% of the total cells, whereas intermediate and parabasal cells came to roughly 65% and 20%, in that order. Under all treatments, there was a noticeable drop in the vaginal pH values Additionally, for all test products, the upper portion of the dose-response curve was achieved for the pH values. As a result, no significant dosage dependency was seen.

Security

This trial did not report any major adverse events. A total of 26 out of 31 participants (83.87%) reported 64 adverse events (AEs) while using of the experimental goods. Eight AEs were determined to have no causal relationship to the medication under test, whereas fifty-six were determined to be drug-related. Of the drug-related adverse events, 18 out of 56 (32.14%) happened

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during Test 1 treatment, 15 out of 56 (26.79%) during Test 2 treatment, and 23 out of 56 (41.07%) during Test 3 treatment. Headache was the most commonly reported adverse event (AE) during the use of the vaginal rings, accounting for 16 out of 56 (28.57%) of the AEs that were determined to be attributable to the administered medication. The evaluations of local tolerability were conducted both before and after the device was removed from each patient. In general, there was one discovery made on local tolerability. After the Test 3 ring was removed, the investigator noticed an adverse event (AE) called vulvovaginal candidiasis. This condition was deemed to be somewhat intense and most likely caused by the test medication. Up to the study's final inspection, the adverse event was managed. As a substitute safety metric, the ET was established None of the participants who took Test 1 saw a rise in ET values greater than 5 mm. In two out of ten participants who received Test 2, the ET rose throughout treatment to levels greater than 5 mm, but it didn't return to the baseline until day 42. Two of the ten individuals who took Test 3 had ET increases above 5 mm throughout therapy and did not return to baseline until day 42. for a single topic. On the other hand, the other participant required the initiation of withdrawal hemorrhage before the ET recovered to baseline (5 mm). None of the three treatments showed any evidence of a dose-dependent effect on ET. Between the screening and study-end exams, no clinically significant changes were seen in the laboratory data, ECG parameters, vital signs, or physical parameters linked to safety.

F-5 Approximate production of estrogens (profile) in women with age. Estrogen levels peak in the late 20s. Estrogen levels during perimenopause fluctuate greatly around a normal range until menopause, when no more responsive follicles are available. In the USA, most women experience menopause from 40 to 58 years of age, with the average at 51 years of age (see red rectangular bar above the

Conversation

The goal of the current investigation was to examine the pharmacokinetics of oestriol at three distinct dosages in a descriptive manner. when taking a single dose. Additionally, the safety and pharmacodynamic effects of these medications were assessed in postmenopausal healthy women. The measurement of oestriol plasma concentrations following the application of three differently dosed vaginal rings, designated as Test 1 (0.125 mg day-1), Test 2 (0.250 mg day 1), and Test 3 (0.500 mg day–1), revealed that, overall, the ring systems' in vivo delivery performance was very consistent. The systemic exposure decline following an initial peak could be partially attributed to the device's release characteristics. But with time, postmenopausal women's systemic exposure to vaginally administered oestrogen decreases because of the trophic impact of the The impact of sexual hormones on vaginal cell proliferation leads to an enhancement in the mucosa's barrier function [13].The in vivo performance and the in vitro release rates were comparable, according to comparison with the in vitro release data. Consistent with the matrix release, However, every product displayed a high rate of release at first, which sharply declined until roughly five days later, at which point a plateau phase was reached. The plateau phase's level depended on the medication concentration. It appears that the in vivo proteins attain this plateau a little bit later. However, it was challenging to draw clear conclusions from the in vitro model on the in vivo release of the intravaginal rings because of the contemporaneous proliferation of the vaginal mucosa. The dose-adjusted values' box plots clearly show dose-proportionality for AUC0-tlast and AUC0-21d values, corroborated by an evaluation taking the power model into account; nevertheless, the increase for Cmax was less than proportionate. The assessment of the FSH and LH baseline values verified that the individuals under investigation were postmenopausal. But the baseline values also showed that the group was not homogeneous, which was also noted in a prospective research that took place one and two years following menopause [1]. When interpreting the data, one must take this restriction into account that was inferred from the population characteristics. In all three test groups, subjects also displayed a rather wide range of SHBG concentrations, with values ranging from 25 to 75 nmol l–1, despite one patient pre-senting 105 nmol l–1. Literature-based data revealed SHBG levels within the postmenopausal state, within the range of 30 to 75 nmol l–1 [1, 21].As dosage increased, there was a more noticeable mean maximum reduction in serum concentrations of LH and FSH. The dosage also had an impact on the size and rate of increase in FSH concentrations; for example, the subsequent increase was less abrupt, increasing gradually and reaching a mean value below baseline on day 19 of 15.8

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mIU ml–1 for Test 3. Moreover, during the second half of the test, the FSH values for only Test 1 returned to baseline duration of the treatment, that is, while receiving treatment. After receiving conventional systemic treatment for 20 weeks, the average FSH readings decreased from 77.1 ± 14.8 mIU ml–1 to 39.8 ± 15.8 mIU ml–1. HRT (oral medroxyprogesterone acetate 5 mg day–1 and transdermal oestradiol, 50 μg day–1) [22]. Therefore, after 20 weeks of treatment, the observed absolute difference in FSH values in a population with comparable baseline values was about 30% lower than that found under Test 3 medication in the current study, where FSH values decreased from 74 mIU ml–1 to about 60 mIU ml–1 after 21 days of treatment.. The average result on day 5 of the relative maximal FSH suppression that was noted during Test 3 is 37%. This result is between the effects of 0.625 mg conjugated oestrogen (18.6 ± 20.6% FSH reduction) and 1.25 mg conjugated oestrogen. It is comparable to the daily dosage of 1 mg piperazine oestrone sulfate.

(55.0 ± 9.8% suppression of FSH) [23].The impression is that the effect on LH was less enduring over the course of the 21-day therapy than the effect on FSH levels, based on the trajectories of the curves over time (Figure 4). Within seven days, rather consistent LH levels have already become close to baseline levels. An rise in SHBG values under treatment was seen for all test groups; however, the increase was very moderate, with the highest occurring in an average magnitude of The typical range was 5–10 nmol l–1 during the second part of the therapy cycle. The pattern inferred from SHBG readings was less evident than that from the decline in FSH and LH because of the wide range of baseline values and the gradual, erratic increase over time. The rise in the SHBG mean curves demonstrated no relationship between dose and baseline, with the largest increase seen for the lowest dose (Test 1), followed by Tests 3 and 2. All curves, however, decreased between days 19 and 22.The percentage SHBG increase in the current study appeared to be less than that shown for equipotent oestrogens, rising from 39 nmol l–1 to roughly 47 nmol l–1 on day 16 of Test 3 [23]. In the event that FSH suppression serves as an anchor for oestrogenic potency markers, FSH depression during Test 3 treatment would fall between the range of 0.625 mg and 1.25 mg of conjugated oestrogen's effects. A 38 nmol l–1 and 54 nmol l–1 SHBG rise, respectively, has been measured at these dosages. This might be a sign of oestriol's reduced hepatic oestrogen action when compared to other oestrogens.Vaginal atrophy was present in the participants, and all of the treatment subjects had an increase in their MV, with a threshold of MV >40%. With all three test doses, the maximal effect of 70–80% on the MV was achieved. As a result, variations in the dosages were not discernible.

Menstrual cycle patterns during menopause. FMP ¼ final menstrual period

The distribution of parabasal, intermediate, and superficial cells indicated a shift towards superficial cells, and the pattern of cellular alterations with respect to the maturation index under treatment was exactly in accordance with the expectations Previous research on MVs has demonstrated similar results. After three weeks of treatment with ultra-low-dose vaginal oestriol, the MVs increased from roughly 40% to an average value of 75%. to females suffering from vaginal atrophy [15]. After three weeks of treatment with a pessary that continually releases oestriol, a comparable increase from 26% up to 61% was also noted [24]. As a result, the results of this investigation demonstrated that, while the upper portion of the dose response curve was reached for all treatments, the effect of all tested items on MV was independent of dose. The reason substantial product changes cannot be discovered is that it is known that MVs above 50 are observed in healthy premenstrual women, meaning that the values observed during treatments were already near to the normal situation.Vaginal pH levels above 4.5 are indicative of the menopausal condition [25]. The pH readings were ≥5 at the beginning The majority of individuals with pH levels greater than 5 also had MVs greater than 40%, and typically higher pH values are associated with vaginal atrophy. As was mentioned for the MV, there was no discernible difference in the greatest effect between the therapies even at the lowest dosage.For each of the three therapies, there was no documented dose-dependent effect on ET. Nonetheless, exposure to estrogen generally results in a rise in ET [26]. In fact, the study's sample size was insufficient to make inferences in this particular instance. All test items had generally good tolerance, which was consistent with oestriol's safety and tolerability profile in prior vaginal formulations. Based on the findings of the safety assessment, there are no unfavorable effects on using the oestriol test medication in a vaginal ring.

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Additionally, the three formulations under investigation demonstrated safety, as seen by the Cmax values of 68, 97, and 155 pg ml–1 for Tests 1, 2, and 3, in that order. These levels are less than the mean plasma concentration reported for women with multiple sclerosis following a 12-month course of oral estriol medication (8 mg day–1) and for healthy pregnant women who did not receive treatment (6 ng ml–1) [16].

F-7 Changes of vagina after the first time of laser therapy.

In summary,

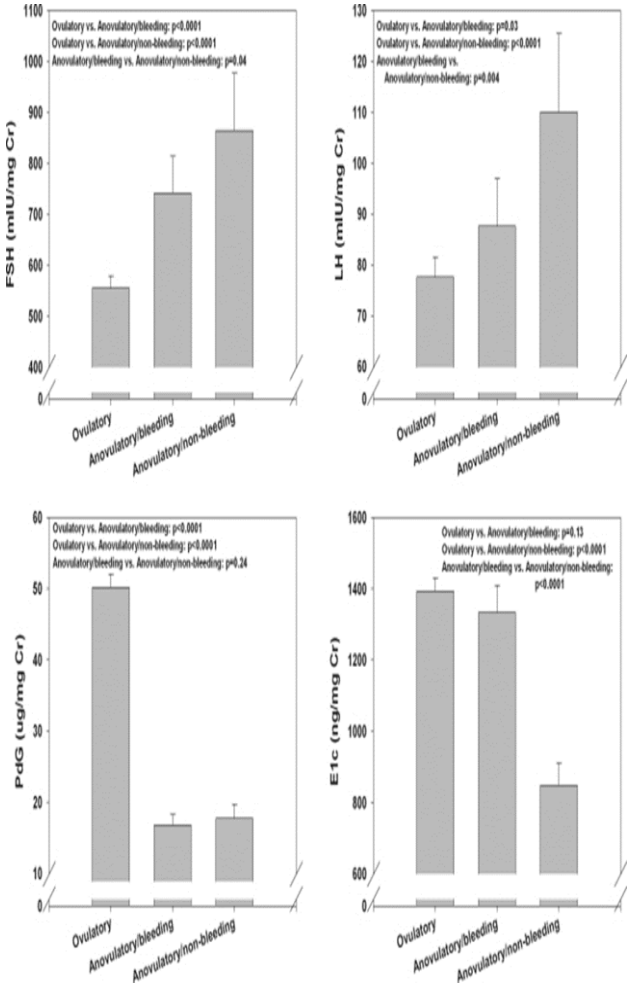
it was anticipated that the MV and pH values would show local impacts on the vagina, while the hormonal status would show systemic exposure. It was challenging to forecast the degree of exposure because the in vivo release rate of the ring system was unknown at the time of study preparation. The regional impacts were taken into account. as distinguishing indicators for low exposure, while the changes in the hormonal system were anticipated to signify significant exposure and enable separation between the tested amounts. Maximum local impact was

triggered by the release of adequate levels of oestriol in all three test formulations. Because of this, it was not possible to evaluate the differences between Tests 1, 2, and 3 using the local pharmacodynamic parameters (pH and MV). For FSH and LH levels, it was evident that there was a dose dependency; however, for SHBG, the conclusion was unclear.

f-3 Study of Women's Health Across the Nation (SWAN). Percent of cycles without evidence of luteal activity (ELA)

Analyses using statistics

Since this clinical trial was a descriptive pilot study, sample size estimation based on statistics has not been carried out. Every treatment's descriptive statistics were assessed for every pharmacokinetic and pharmacodynamic parameter. AUC0-tlast, AUC0-∞, and Cmax were calculated using the power model as outlined by Gough et al. [18] in order to evaluate dosage proportionality. Terms used to refer to targets and ligands Important protein targets and ligands. The overall analysis set contained thirty-one participants, while the per-protocol set contained thirty (PPS). One participant left the research on day six after withdrawing their consent, while the other due to a day 19 procedure infraction. For the second subject, the data for the pertinent study variables were nearly finished, therefore she was added to the PPS. Data from the participants included in the PPS were used for pharmacokinetic and pharmacodynamic evaluation (n = 10 for each medication). Data from the subjects included in the entire analytic set were used for safety evaluation, demographics and other baseline characteristics Medication The in vitro initial release rate of the oestriol-containing vaginal rings was high and declined markedly until approximately After five days, a plateau phase was attained. Drug concentration determined the elevation of the plateau phase For Test 1, 2, and 3, the mean release rates were 0.165 mg day–1 (ranging from an average of 0.666 mg day–1 on day 1 to 0.086 mg on day 21), 0.318 mg day–1 (ranging from an average of 1.449 mg day–1 on day 1 to 0.164 mg on day 21), and 0.667 mg day–1 (ranging from an average of 3.470 mg day–1 on day 1 to 0.331 mg on day 21). After the in vitro–in vivo correlation was carried out, 0.999872184 was the correlation coefficient.



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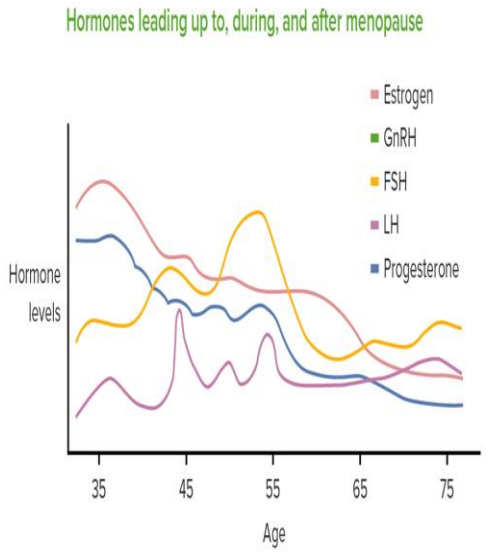


Menarche — FMP (0)

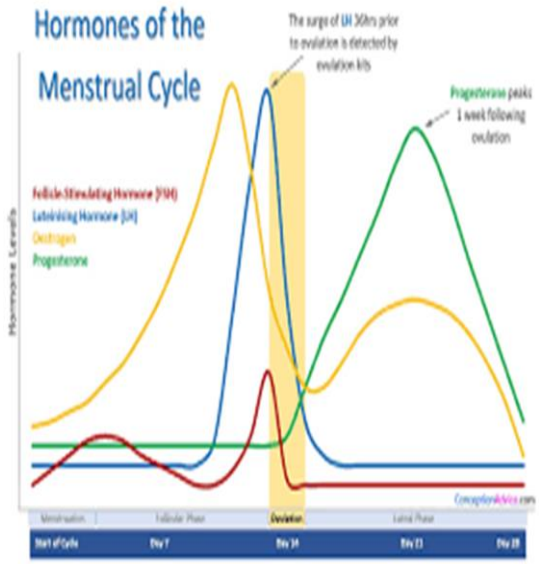
Stage	-5	-4	-3b	-3a	-2	-1	+1 a	+1b	+1c	+2
Terminology	REPRODUCTIVE				MENOPAUSAL TRANSITION		POSTMENOPAUSE			
	Early	Peak	Late		Early	Late	Early			Late
					Perimenopause					
Duration	*variable*				*variable*	1-3 years	2 years (1+1)	3-6 years		*Remaining lifespan*
PRINCIPAL CRITERIA										
Menstrual Cycle	Variable to regular	Regular	Regular	Subtle changes in Flow/ Length	*Variable Length* Persistent ≥7- day difference in length of consecutive cycles	Interval of amenorrhea of >=60 days				
SUPPORTIVE CRITERIA										
Endocrine FSH AMH Inhibin B			Low Low	Variable* Low Low	↑ Variable* Low Low	↑ >25 IU/L** Low Low	↑ Variable Low Low	Stabilizes Very Low Very Low		
Antral Follicle Count			Low	Low	Low	Low	Very Low	Very Low		
DESCRIPTIVE CHARACTERISTICS										
Symptoms						Vasomotor symptoms *Likely*	Vasomotor symptoms *Most Likely*			*Increasing* symptoms of urogenital atrophy

* Blood draw on cycle days 2-5 ↑ = elevated

**Approximate expected level based on assays using current international pituitary standard[67-69]

Pharmacodynamics

serum levels of FSH, LH, and SHBG. Time curves illustrating the mean serum concentration for variations from baseline in FSH, LH, and is SHBG during vaginal application of one vaginal ring of Test 1, Test 2, or Test 3. Every individual exhibited baseline values beyond 20 mIU l–1 for FSH and above 30 mIU l–1 for LH , which are widely recognized as threshold values for the postmenopausal condition. The box plots that while SHBG values gradually increased until day 19, the highest effect for LH and FSH values was obtained on days 2 and 5, respectively, after ring implantation. MV and pH in the vagina. For every therapy, the MV changes were almost the same The modifications began to disappear 21 days following treatment termination. Similar results were seen in the cytology of the vaginal smear (parabasal, intermediate, and superficial cells), which has been described in order to determine the MV The three treatment groups' mean baseline values before the start of the intervention were very comparable, with roughly 50% of the cells being parabasal, 40% being intermediate, and 10% being specialized. Following 21 days (day 22) of therapy, the percentage of superficial cells in the sample was, on average, around 50%. All other cells were classified as intermediate cells; no parabasal cells were found in the samples. Day 42: After 21 days in a row without therapy, superficial cells made up approximately 15% of the total cells, whereas intermediate and parabasal cells came to roughly 65% and 20%, in that order. Under all treatments, there was a noticeable drop in the vaginal pH values Additionally, for all test products, the upper portion of the dose-response curve was achieved for the pH values. As a result, no significant dosage dependency was seen.



Security

This trial did not report any major adverse events. A total of 26 out of 31 participants (83.87%) reported 64 adverse events (AEs) while using of the experimental goods. Eight AEs were determined to have no causal relationship to the medication under test, whereas fifty-six were determined to be drug-related. Of the drug-related adverse events, 18 out of 56 (32.14%) happened during Test 1 treatment, 15 out of 56 (26.79%) during Test 2 treatment, and 23 out of 56 (41.07%) during Test 3 treatment. Headache was the most commonly reported adverse event (AE) during the use of the vaginal rings,

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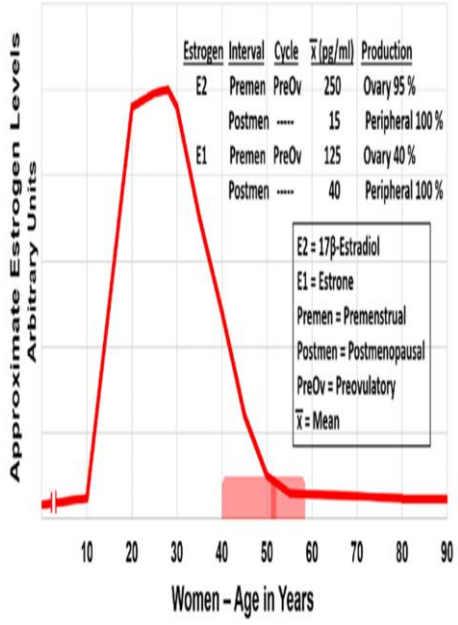
accounting for 16 out of 56 (28.57%) of the AEs that were determined to be attributable to the administered medication. The evaluations of local tolerability were conducted both before and after the device was removed from each patient. In general, there was one discovery made on local tolerability. After the Test 3 ring was removed, the investigator noticed an adverse event (AE) called vulvovaginal candidiasis. This condition was deemed to be somewhat intense and most likely caused by the test medication. Up to the study's final inspection, the adverse event was managed. As a substitute safety metric, the ET was established None of the participants who took Test 1 saw a rise in ET values greater than 5 mm. In two out of ten participants who received Test 2, the ET rose throughout treatment to levels greater than 5 mm, but it didn't return to the baseline until day 42. Two of the ten individuals who took Test 3 had ET increases above 5 mm throughout therapy and did not return to baseline until day 42. for a single topic. On the other hand, the other participant required the initiation of withdrawal hemorrhage before the ET recovered to baseline (5 mm). None of the three treatments showed any evidence of a dose-dependent effect on ET. Between the screening and study-end exams, no clinically significant changes were seen in the laboratory data, ECG parameters, vital signs, or physical parameters linked to safety.



F-5 Approximate production of estrogens (profile) in women with age. Estrogen levels peak in the late 20s. Estrogen levels during perimenopause fluctuate greatly around a normal range until menopause, when no more responsive follicles are available. In the USA, most women experience menopause from 40 to 58 years of age, with the average at 51 years of age (see red rectangular bar above the

Conversation

The goal of the current investigation was to examine the pharmacokinetics of oestriol at three distinct dosages in a descriptive manner. when taking a single dose. Additionally, the safety and pharmacodynamic effects of these medications were assessed in postmenopausal healthy women. The measurement of oestriol plasma concentrations following the application of three differently dosed vaginal rings, designated as Test 1 (0.125 mg day-1), Test 2 (0.250 mg day 1), and Test 3 (0.500 mg day–1), revealed that, overall, the ring systems' in vivo delivery performance was very consistent. The systemic exposure decline following an initial peak could be partially attributed to the device's release characteristics. But with time, postmenopausal women's systemic exposure to vaginally administered oestrogen decreases because of the trophic impact of the The impact of sexual hormones on vaginal cell proliferation leads to an enhancement in the mucosa's barrier function [13].The in vivo performance and the in vitro release rates were comparable, according to comparison with the in vitro release data. Consistent with the matrix release, However, every product displayed a high rate of release at first, which sharply declined until roughly five days later, at which point a plateau phase was reached. The plateau phase's level depended on the medication concentration. It appears that the in vivo proteins attain this plateau a little bit later. However, it was challenging to draw clear conclusions from the in vitro model on the in vivo release of the intravaginal rings because of the contemporaneous proliferation of the vaginal mucosa. The dose-adjusted values' box plots clearly show dose-proportionality for AUC0-tlast and AUC0-21d values, corroborated by an evaluation taking the power model into account; nevertheless, the increase for Cmax was less than proportionate. The assessment of the FSH and LH baseline values verified that the individuals under investigation were postmenopausal. But the baseline values also showed that the group was not homogeneous, which was also noted in a prospective research that took place one and two years following menopause [1]. When interpreting the data, one must take this restriction into

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account that was inferred from the population characteristics. In all three test groups, subjects also displayed a rather wide range of SHBG concentrations, with values ranging from 25 to 75 nmol l–1, despite one patient pre-senting 105 nmol l–1. Literature-based data revealed SHBG levels within the postmenopausal state, within the range of 30 to 75 nmol l–1 [1, 21].As dosage increased, there was a more noticeable mean maximum reduction in serum concentrations of LH and FSH. The dosage also had an impact on the size and rate of increase in FSH concentrations; for example, the subsequent increase was less abrupt, increasing gradually and reaching a mean value below baseline on day 19 of 15.8 mIU ml–1 for Test 3. Moreover, during the second half of the test, the FSH values for only Test 1 returned to baseline duration of the treatment, that is, while receiving treatment. After receiving conventional systemic treatment for 20 weeks, the average FSH readings decreased from 77.1 ± 14.8 mIU ml–1 to 39.8 ± 15.8 mIU ml–1. HRT (oral medroxyprogesterone acetate 5 mg day–1 and transdermal oestradiol, 50 μg day–1) [22]. Therefore, after 20 weeks of treatment, the observed absolute difference in FSH values in a population with comparable baseline values was about 30% lower than that found under Test 3 medication in the current study, where FSH values decreased from 74 mIU ml–1 to about 60 mIU ml–1 after 21 days of treatment.. The average result on day 5 of the relative maximal FSH suppression that was noted during Test 3 is 37%. This result is between the effects of 0.625 mg conjugated oestrogen (18.6 ± 20.6% FSH reduction) and 1.25 mg conjugated oestrogen. It is comparable to the daily dosage of 1 mg piperazine oestrone sulfate.

(55.0 ± 9.8% suppression of FSH) [23].The impression is that the effect on LH was less enduring over the course of the 21-day therapy than the effect on FSH levels, based on the trajectories of the curves over time (Figure 4). Within seven days, rather consistent LH levels have already become close to baseline levels. An rise in SHBG values under treatment was seen for all test groups; however, the increase was very moderate, with the highest occurring in an average magnitude of The typical range was 5–10 nmol l–1 during the second part of the therapy cycle. The pattern inferred from SHBG readings was less evident than that from the decline in FSH and LH because of the wide range of baseline values and the gradual, erratic increase over time. The rise in the SHBG mean curves demonstrated no relationship between dose and baseline, with the largest increase seen for the lowest dose (Test 1), followed by Tests 3 and 2. All curves, however, decreased between days 19 and 22.The percentage SHBG increase in the current study appeared to be less than that shown for equipotent oestrogens, rising from 39 nmol l–1 to roughly 47 nmol l–1 on day 16 of Test 3 [23]. In the event that FSH suppression serves as an anchor for oestrogenic potency markers, FSH depression during Test 3 treatment would fall between the range of 0.625 mg and 1.25 mg of conjugated oestrogen's effects. A 38 nmol l–1 and 54 nmol l–1 SHBG rise, respectively, has been measured at these dosages. This might be a sign of oestriol's reduced hepatic oestrogen action when compared to other oestrogens.Vaginal atrophy was present in the participants, and all of the treatment subjects had an increase in their MV, with a threshold of MV >40%. With all three test doses, the maximal effect of 70–80% on the MV was achieved. As a result, variations in the dosages were not discernible.

The distribution of parabasal, intermediate, and superficial cells indicated a shift towards superficial cells, and the pattern of cellular alterations with respect to the maturation index under treatment was exactly in accordance with the expectations Previous research on MVs has demonstrated similar results. After three weeks of treatment with ultra-low-dose vaginal oestriol, the MVs increased from roughly 40% to an average value of 75%. to females suffering from vaginal atrophy [15]. After three weeks of treatment with a pessary that continually releases oestriol, a comparable increase from 26% up to 61% was also noted [24]. As a result, the results of this investigation demonstrated that, while the upper portion of the dose response curve was reached for all treatments, the effect of all tested items on MV was independent of dose. The reason substantial product changes cannot be discovered is that it is known that MVs above 50 are observed in healthy premenstrual women, meaning that the values observed during treatments were already near to the normal situation.Vaginal pH levels above 4.5 are indicative of the menopausal condition [25]. The pH readings were ≥5 at the beginning The majority of individuals with pH levels greater than 5 also had MVs greater than 40%, and typically higher pH values are associated with vaginal atrophy. As was mentioned for the MV, there was no discernible difference in the greatest effect between the therapies even at the lowest dosage.For each of the three

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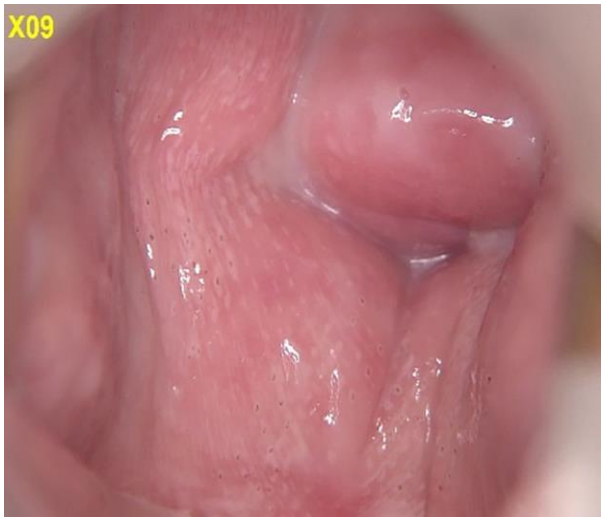
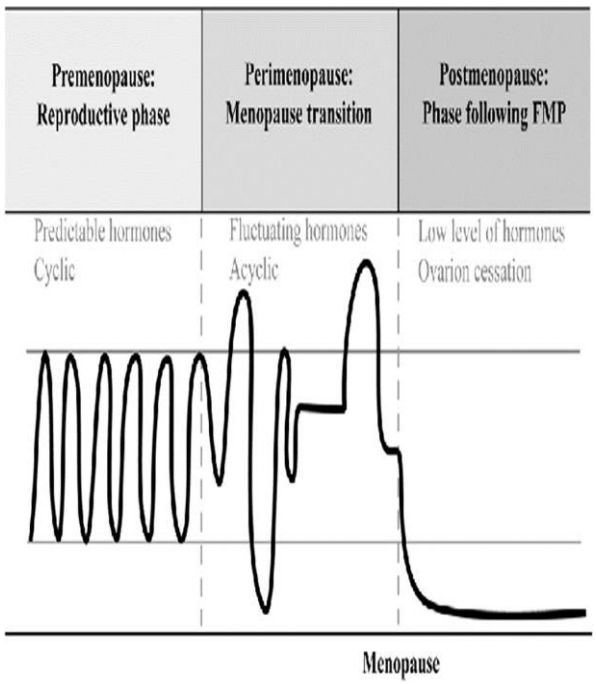
therapies, there was no documented dose-dependent effect on ET. Nonetheless, exposure to estrogen generally results in a rise in ET [26]. In fact, the study's sample size was insufficient to make inferences in this particular instance. All test items had generally good tolerance, which was consistent with oestriol's safety and tolerability profile in prior vaginal formulations. Based on the findings of the safety assessment, there are no unfavorable effects on using the oestriol test medication in a vaginal ring.

Additionally, the three formulations under investigation demonstrated safety, as seen by the Cmax values of 68, 97, and 155 pg ml–1 for Tests 1, 2, and 3, in that order. These levels are less than the mean plasma concentration reported for women with multiple sclerosis following a 12-month course of oral estriol medication (8 mg day–1) and for healthy pregnant women who did not receive treatment (6 ng ml–1) [16].



F-7 Changes of vagina after the first time of laser therapy.



In summary,

it was anticipated that the MV and pH values would show local impacts on the vagina, while the hormonal status would show systemic exposure. It was challenging to forecast the degree of exposure because the in vivo release rate of the ring system was unknown at the time of study preparation. The regional impacts were taken into account. as distinguishing indicators for low exposure, while the changes in the hormonal system were anticipated to signify significant exposure and enable separation between the tested amounts. Maximum local impact was triggered by the release of adequate levels of oestriol in all three test formulations. Because of this, it was not possible to evaluate the differences between Tests 1, 2, and 3 using the local pharmacodynamic parameters (pH and MV). For FSH and LH levels, it was evident that there was a dose dependency; however, for SHBG, the conclusion was unclear.

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