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Review

Intravaginal Drug Delivery Systems to Treat the Genitourinary Syndrome of Menopause: Towards the Design of Safe and Efficacious Estrogen-loaded Prototypes

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ABSTRACT

Estrogens locally delivered to the vagina by tablets, capsules, rings, pessaries, and creams are the most common and highly recommended platforms to treat the genitourinary syndrome of menopause (GSM). Estradiol, an essential estrogen, is routinely administered alone, or in combination with progestins, to effectively alleviate the symptoms associated with moderate to severe menopause when non-pharmacological interventions are not indicated. Since the risk and side effects of estradiol use depends on the administered amount and duration of use, the lowest effective dose of estradiol is recommended when long-term treatment is required. Although there is a wealth of data and literature comparing vaginally administered estrogen-containing products, there is a lack of information revealing the effect of the delivery system used and formulation constituent's attributes on the efficacy, safety, and patient acceptability of these dosage forms. This review therefore aims to classify and compare various designs of commercially available and non-commercial vaginal 17β-estradiol formulations and analyze their performance in terms of systemic absorption, efficacy, safety, and patient satisfaction and acceptance. The vaginal estrogenic platforms included in this review are the currently marketed and investigational 17β-estradiol tablets, softgel capsules, creams, and rings for the treatment of GSM, based on their different design specifications, estradiol loads, and materials used in their preparation. Additionally, the mechanisms of the effects of estradiol on GSM have been discussed, as well as their potential impact on treatment efficacy and patient compliance.

Introduction

The genitourinary symptoms experienced during menopause are broad and include a number of physiological processes that often result in dryness of the vagina, pain during intercourse, urinary inconsistency and hot flashes.[1] In 2014, the term of genitourinary syndrome of menopause (GSM) was introduced for the first time, replacing the terms vulvovaginal atrophy, vaginal atrophy, and urogenital atrophy, with both postmenopausal and premenopausal women affected by this condition. However, GSM has been noted to be more prevalent in postmenopausal women,[2,3] with more than 50% experiencing at least one of the above-mentioned symptoms.[4-7] Despite the high prevalence and availability of treatments for GSM, these conditions are often under-diagnosed and ineffectively treated for several reasons including: the unwillingness of women to discuss the symptoms with their healthcare provider, a lack of education regarding the condition and its treatments, women not recognizing that their experienced symptoms relate to estrogenic deficiency, and many healthcare practitioners not assessing their patients for GSM symptoms.[8-10]

The initial aim of treating GSM symptoms is to lessen and eliminate the experienced symptoms.[3] Numerous treatment options are available for GSM and can be classified into non-hormonal therapy and hormone replacement therapy. Non-hormonal therapy is considered the first-line treatment for mild symptoms and includes vaginal lubricants and moisturizers. Recently, ospemifene, laser therapy, and radio frequency-based therapy are also being used as new alternatives when estrogenic therapies are not recommended. It is routinely only when severe and persistent symptoms are experienced, and non-hormonal therapy is not as effective, that hormonal treatment, including estrogen-containing medications, are introduced. They are considered the "gold standard" pharmacological treatment and the most effective therapy since they act directly towards the cause of GSM; the lack of estrogen.[1,3,11]

Several routes of administration are used for delivering estrogen-containing medications, including the vaginal, oral or transdermal

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routes.[3,12] Oral and transdermal delivered estrogens, however, face numerous problems and limitations, such as first-pass metabolism, the need for frequent administration, and unsteady estrogen plasma concentrations.[13] The vaginal route, however, exhibits superior outcomes than other routes; due to the vagina possessing a mucous membrane with a large surface area, an abundance of blood vessels, bypassing first-pass hepatic metabolism, and the ability to retain a high amount of drug locally.[3,14-17] Using estrogen-containing medications, mainly through the vaginal route, also leads to the restoration of the vaginal cytology with increased superficial cells, raised vaginal blood flow and fluid secretions, maintenance of the microbiological balance of the vagina, reduced pH, increased resistance of the vaginal cell to infection and inflammation, and as a consequence, improvement in GSM symptoms.[9,18,19]

Drug distribution throughout the vaginal tissue differs noticeably with the attributes of the delivery system, with the selection of the vaginal delivery system often relying on the intended effect of treatment. Drug retention at the mucosal surface with even distribution and low mucosal penetration is favored for local effects, while a systemic effect could be obtained via controlled extended drug absorption through the vaginal epithelium. Semi-solid systems are also desirable for local effects because they distribute evenly in the vaginal cavity and are bio-adhesive, while vaginal ring systems are more suitable for topical effects.[17,20] Various vaginal estradiol-preparations are currently on the market and are in use for the local treatment of GSM, including creams (Estrace®), tablets (Vagifem®), and vaginal rings (Estring® and Femring®), which beside its local effects, also exert systemic effects and are used in the treatment of hot flashes.[21,22]

Although estradiol vaginal delivery systems have been revealed to be efficient, they differ in their components and there are concerns about the properties of some of these systems. These vaginal preparations, additionally, have several limitations and drawbacks, such as messiness, a need for an applicator, low retention times and frequent insertions required for tablets and creams.[23-31] Furthermore, the rings are invasive during their insertion and removal, may dislodge, and have problems associated with the initial burst release,[32] and though the performances of the vaginally-administered estrogen platforms in terms of efficacy, safety, and tolerance have been thoroughly researched, there is limited data correlating this performance to their design attributes. Therefore, the purpose of this review is to describe and compare various vaginal 17β-estradiol formulations based on their design specifications, loaded amount of estradiol, and carrier, as well as to evaluate their performance in terms of systemic absorption, efficacy, safety, and patient acceptability.

Pathophysiological Considerations of GSM for Intravaginal System Design

GSM depicts the wide range of changes that occur due to the decreased level of estrogen associated with menopausal transition,[1,3,11,12,33] or due to an iatrogenic estrogen deficiency during reproductive age resulting from external factors, such as drug use, oophorectomy, and cancer treatment.[34] This decreased level of estrogen leads to a breakdown of collagen and elastin fibers, resulting in genital changes such as the loss of elasticity, labia minora fading, labia majora shortening, narrowing of the introital opening, vaginal shrinkage and a narrowing and thin-pale vaginal epithelium.[19,34] Also, the decline of estrogen level is associated with the reduction of blood flow to the vagina, causing less exudation and lubrication during sexual arousal and consequently results in dyspareunia and postcoital bleeding.[19] In addition, glycogen production decreases with the diminished estrogen level, leading to increased pH and imbalance of the normal vaginal microflora, resulting in increased risk of urinary tract infections.[19,35] The gynecological, urological, and sexual signs and symptoms associated with GSM are illustrated in Fig. 1. In general, GSM develops later than the other symptoms of menopause and does not begin until the amount of endogenously produced estrogen is significantly lower than that is essential for endometrial stimulation.[36,37] At this phase of menopause, there is a therapeutic index (Fig. 2) during which it is probable to combat genitourinary atrophy by using lower doses of estrogen than that normally prescribed, without hazardous endometrial hyperplasia.[36]

Menopause-specific Factors Affecting Drug Absorption from the Vagina

The onset of menopause has numerous effects on the absorption of drugs, not only in the treatment of menopause. Drug absorption from the vagina occurs in two major steps: i) drug dissolution within the vaginal cavity, and ii) permeation through the vaginal membrane, which occurs by the transcellular or paracellular pathways. Thus, any elements that influence these steps may impact drug absorption patterns from the vagina.[17,20] These elements may include formulation characteristics, physicochemical properties of the drug and the excipients used, and the physiological features of the vagina.[17,38]

Aligned Physicochemical Properties of Drug and Excipients for Intravaginal Delivery

The vaginal absorption of a drug can be affected by its properties such as its hydrophobicity, molecular weight, surface charge, and ionization. Hydrophilic steroids (such as hydrocortisone) show lower absorption through the vaginal mucosa compared to lipophilic steroids (progesterone/estrone). Additionally, high molecular weight lipophilic therapeutic agents are less likely to be absorbed trans-vaginally than those with a lower molecular weight.[20] Weakly basic drugs with a pKa of 8.5-10.5 are also readily ionized in the low pH vaginal fluid of productive-age women, while weakly acidic drugs with a pKa of less than 5.5 remain unionized. Nonetheless, the oscillating vaginal pH can directly influence the ionization of a drug, and consequently drug solubility and absorption.[17]

The characteristics of the excipients and delivery system used can also greatly influence drug absorption (e.g., vaginal rings and tablets exhibit less systemic drug exposure than creams). The intended therapeutic outcome could therefore be achieved through the appropriate design of the delivery system.[17,38] Additionally, through the proper selection of excipients, it is possible to control the hydrophilic-lipophilic balance and the extent of absorption as a consequence, making the pKa of the drug of more importance than its molecular weight in obtaining a formulation with the desired characteristics.[17]

17β-estradiol belongs to Class II in the Biopharmaceutical Classification System with a poor water solubility (0.2– 5 μg/mL) and a high permeability.[39] It is well absorbed orally[40,41] and undergoes broad first pass metabolism, leading to poor bioavailability. As a consequence, large and frequent oral doses are often required, leading to greater side effects.[40,42,43] 17β-estradiol also exists commercially in different dosage forms including oral, vaginal, and transdermal formulations.[42,44,45] However, the physicochemical properties of the drug can be hindered by the dynamic changes in the vaginal environment, hence, it is critical to evaluate these physiological conditions prior to developing such delivery systems.[17]

Salient Physiologic Features for Optimal Intavaginal Drug Absorption

Drug absorption through the vaginal mucosa is influenced by the physiological conditions of the vagina including the mucosal thickness, vaginal fluid pH and viscosity, hormonal levels changes and the presence of enzymes.[38]

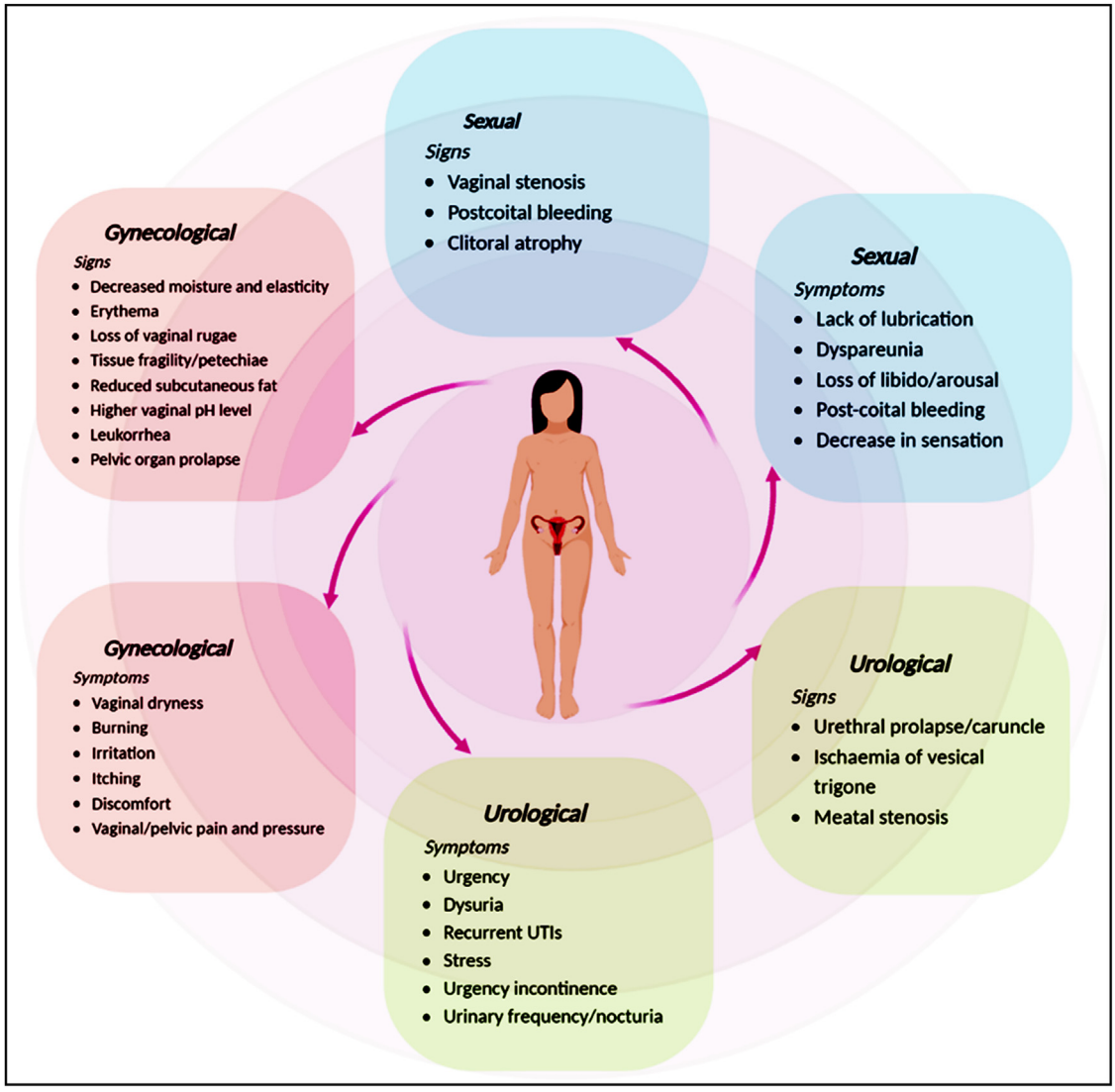

Figure 1. The common clinical manifestations of GSM.

Vaginal Fluid Volume and pH

The vaginal fluid volume and pH are both important parameters that influence the absorption of vaginally administered drugs.[46] Because the vaginal epithelium lacks secretory glands, vaginal fluid is primarily a mixture of several components sourced from blood vessel transudates and contributions from the upper reproductive tract fluids, Bartholin's and Skene's glands, and exfoliated epithelial cells.[20,46-49] The reported amount of vaginal fluid over 24 hours is highly variable with studies reporting 1-3g, 6g and 7.92g per day, with 0.5−0.75 mL continuously present in the vagina at any given time.[48,49] The aqueous nature of vaginal fluid is essential for drug solubilization,[46] and the large amounts of vaginal fluid can alter the muco-adhesive and rheological properties of the drug formulation, resulting in low retention time and consequently washout of the dosage form.[17,46]

In healthy reproductive-aged women, estrogen also induces glycogen production, which is converted by *Lactobacilli* into lactic acid, leading to the maintenance of an acidic vaginal pH (3.5-4.5). During menopause and other conditions resulting in decreased estrogen levels, vaginal glycogen and *Lactobacilli* decrease, leading to a rise of vaginal pH (>5).[34,50] This change in vaginal pH directly affects the degree of drug ionization, affecting drug absorption.[17] In addition, the microbiome imbalance in the vagina during menopause can lead to the formation of a layer of pathogenic bacteria on the vaginal mucosa, which can act as an obstacle to the absorption and/or local action of a drug.[17]

Vaginal Tissue Dynamics

The vaginal wall comprises of three layers; the adventitia, muscularis, and mucosa, which is composed of non-secretory epithelia.[20,46] The maturation and thickness of the vaginal epithelium is controlled by sex hormones. In adult premenopausal women, the normal thickness of the epithelium ranges between 200-300 mm.[46] After menopause and other hypo-estrogenic states, the thickness of the vaginal epithelium layer decreases, with the vagina also becoming narrower and shorter.[37,51] The absorption of drugs administered via the vaginal route is therefore directly affected by the alteration of vaginal mucosa characteristics.[17] The thinner atrophic vaginal mucosa also demonstrates a higher absorption rate for estrogen and steroids when compared to non-atrophic mucosa.[20,46]

Enzymatic Activity

Drug absorption and stability of a delivery system can be influenced by the activity of enzymes that are present within the

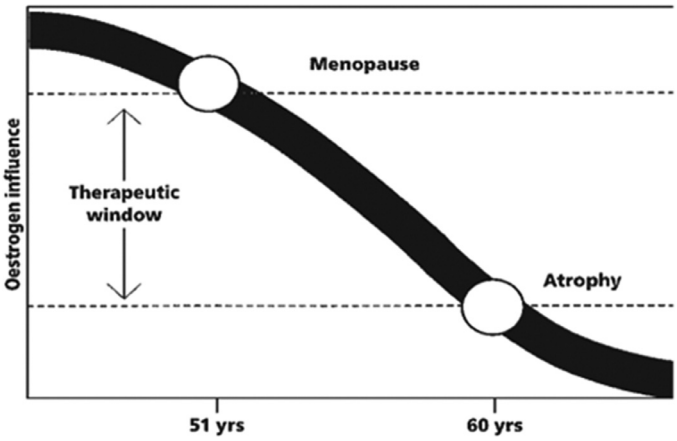

Figure 2. Therapeutic index during which it is probable to combat GSM using lower doses of estrogen. Reproduced with permission from Samsioe[36] © 1998 Mosby, Inc.

vagina.[17,46] A broad range of enzymes exists in the vagina despite the fact that the activity of the enzymes is lower compared to that of the gastrointestinal tract. Vaginal fluid contains enzymes, such as lysozyme, nucleases, aldolase, β-glucuronidase, and esterase. Phosphatases, β-glucuronidase, aminopeptidase, and lactate dehydrogenase furthermore exist in the vaginal mucosa, all of which have the potential to influence drug stability and absorption.[46]

Factors Influencing GSM Severity and Response to Therapy

Demographic factors have been noted to potentially influence the degree of GSM symptom severity, as well as age and body mass index. It is recognized that the proceeding in age leads to lessening estrogen levels, in contrast to an increase of body mass index which leads to an increase in circulating estrogen. Hence, alteration in such factors would expect a change in the severity of GSM symptoms and their response to treatment.[52]

In the 12 week, multi-center, phase 3 REJOICE trial carried out by Constantine et al.,[52] assessment of the influence of age, body mass index, pregnancy and uterine status on the clinical efficacy and safety of 4, 10 and 25 μg doses of TX-004HR was evaluated. The vaginal pH, dyspareunia severity, superficial cells and parabasal cells percentages were analyzed to measure the efficacy outcomes from baseline to week 12 of the different doses, in comparison with a placebo within pre-specified subgroups (age: ≤56, 57 to 61, and ≥62 years, body mass index: ≤24, 25 to 28, and ≥29 kg/m^2, pregnancy history, the number of vaginal births and uterine status: intact or not intact uterus). The findings showed that the three doses significantly lessened dyspareunia, the pH of the vagina and parabasal cells, and increased superficial cells, with an improvement in dyspareunia severity noted as early as Week 2 with a constant effect through the 12 weeks of study.

The frequency and intensity of most GSM symptoms differ according to the time passed since menopause. Sixty five percent of women suffer from GSM symptoms within one-year post-menopause, increasing to 84% within six years.[3] The British Menopause Society and the International Menopause Society recommended that vaginal atrophy treatment be commenced early to obtain the best response.[53] Recently, Derzko et al.[53] analyzed data obtained through a multi-center, randomized, double-blind, parallel-group, placebo-controlled trial and detailed the influence of postmenopausal women age at the start of treatment, using 10 μg Vagifem® as the treatment response. The participants were classified according to their age, younger (≥ 45 - < 60 years) or older (≥ 60 years) and the change from baseline to Week 52 in pH, vaginal maturation index, with the most problematic symptoms evaluated and analyzed. Although a more robust response had been observed in younger women, the findings demonstrated that starting the treatment at any age can improve GSM symptoms. Another study by Simon et al.[54] also looked at the effects of body position (supine and ambulatory or seated) on estradiol bioavailability after administration of 25 μg TX-004HR. C_{max}, $AUC_{0\text{-}24}$, and T_{max} were measured by high-performance liquid chromatography-tandem mass spectroscopy. The findings demonstrated there is no significant difference in bioavailability due to body position after TX-004HR dosing.

Current Estradiol-loaded Vaginal Drug Delivery Systems

The vagina is an important site for local and systemic drug delivery and is considered an effective alternative route to deliver drugs with poor oral bioavailability.[24,55] Vaginal delivery furthermore reduces systemic adverse effects and offers local drug accumulation.[56] It also demonstrates numerous other benefits such as self and easy administration, high permeability, and a large perfusion surface area.[15,56,57] One clinical challenge associated with vaginal dosage forms, however, is patient perspective, as many patients fear that the formulation will be washed out.[58] Traditionally, the vaginal route has been employed to deliver hormones and anti-infective agents such as antivirals, antifungals, and antibacterials, in different dosage forms.[23,57] Currently, various delivery systems for both local and systemic action are available on the market or in clinical development. These systems include classical dosage forms including creams, ointments, pessaries, tablets, and capsules. Recently, novel formulations such as films and vaginal rings have also been developed.[15,25,59] Low dose vaginal estrogen-loaded formulations have also been extensively used due to its avoidance of hepatic metabolism and a greater respond-ability of vaginal tissues from the locally applied estrogen, making these systems considered to be more efficient than systemic delivery in the alleviation of GSM.[60-62] Estradiol-based pessaries and creams were the first vaginal formulations effectively used in relieving the urogenital symptoms of menopause, but they are rarely used for an extended treatment duration.

The choice of the delivery system is therefore highly dependent on the patient's preferences and needs.[63] The dose regimen for the rings is that one ring is inserted vaginally for three months, while dosing for creams and tablets consist of starting with a daily application for two weeks, as a loading dose, followed by applications twice per week as a maintenance dose for the period needed to control symptoms.[7,64] The underlying rationale for this schedule is that during the initial days of therapy, the vaginal epithelium is atrophic, leading to high estradiol absorption. The vaginal epithelium subsequently matures with estradiol use and time, resulting in decreased absorption; hence, smaller doses are usually required to provide adequate estradiol accumulation to avoid vaginal atrophy recurrence.[64] Table 1 shows the FDA approved vaginal estradiol delivery systems, their excipients, estradiol loads, release rates, and their dose regimens, while Table 2 summarizes studies that compare test estradiol vaginal formulations versus placebos, or to other vaginal estrogen formulations.

Vaginal Tablets

Vaginal tablets are characterized by their ease of manufacture and administration, making them more convenient for patients.[102] Vaginal estradiol tablets are approved and marketed commercially for the treatment and alleviation of symptoms associated with atrophic vaginitis and include Vagifem® tablets (Novo Nordisk, Baegsvard, Denmark).[103] Vagifem® tablets are a film-coated hydrophilic cellulose matrix with the ability of adhesion onto the vaginal mucosa and offers controlled in site, pH-independent release with no significant systemic absorption of estradiol.[60,83,90] Vagifem® is present in two

Table 1
FDA approved vaginal estradiol delivery systems.

Delivery system	Active	Generic	Additives	Company	Strength/ Release rate	Dosing	Reference
Cream	17β-estradiol	Estrace	A non-liquefying base containing propylene glycol, stearyl alcohol, white ceresin wax, mono- and diglycerides, hypromellose 2208 (4000 cps), sodium lauryl sulfate, methylparaben, edetate di-sodium, tertiary butylhydroquinone and purified water	Warner Chilcott Laboratories Rockaway, NJ. Allergan USA, Inc., Irvine, CA	0.01%	2-4 g/daily for 1-2 weeks followed by 1-2 g for 1-2 weeks; 1 g maintenance dose 1-3 times weekly.	5,6, 21,63, 65-67
Tablet	Estradiol	- Vagifem - Yuvafem (generic)	Lactose, starch, hydroxyl propyl methyl cellulose (HPMC), magnesium stearate, polyethylene glycol	Novo Nordisk Pharmaceuticals, Princeton, NJ	10 μg/Tablet 25 μg/tablet (discontinued)	10 μg inserts daily for 2 weeks then 2 per week	5,21, 63,65 66,68, 69
Softgel capsules	17β-estradiol	Imvexxy	MIGLYOL 812 N, gelatin, polyethylene glycol stearate, lecithin, propylene glycol, FD&C Red #40, titanium dioxide, glycerine, polyvinyl acetate phthalate	Therapeutics MD, Inc., Boca Raton, FL.	4, 10 μg/insert	4 μg-10 μg inserts daily for 2 weeks then 2 per week	5,68, 70-72
Ring	17β-estradiol	Estring	Silicone polymers and barium sulfate	Pharmacia & Upjohn Pfizer, New York, NY	2 mg 7.5 μg/day	One ring inserted every 3 months	5,21, 63,65 66,68, 73-75
Ring	17β-estradiol-3-acetate	Femring	Cured silicone elastomer composed of dimethyl polysiloxane silanol, silica (diatomaceous earth), normal propyl orthosilicate, stannous octoate; barium sulfate	Warner Chilcott Laboratories, Rockaway, NJ Millicent Pharma	12.4, 24.8 mg 50,100 μg/day	One ring inserted every 3 months	21,65, 73,74, 76

strengths: 10 μg and 25 μg. In 1988 the Vagifem® tablet with the 25 μg 17β-estradiol load was introduced for the first time,[21] however in July 2010, the manufacturing company stopped trading this product in the USA.[80]

The 10 μg Vagifem® tablet was approved by the US Food and Drug Administration (US FDA) for the treatment of vulvovaginal atrophy in November 2009.[21,80] The Vagifem® 10 μg tablet is the lowest approved dose, which has only 1.14 mg estradiol exposure throughout a twelve month period.[104-106] The dose schedule for Vagifem® consists of an application of one tablet per day during the initial two weeks of treatment, followed by one tablet twice a week as a maintenance dosage.[21] Each one Vagifem® tablet is located in a prefilled single-use applicator[83,107] positioned deeply in the vagina; where the tablet adheres to the vaginal mucosa, forming a gel layer from which the estradiol is slowly released by diffusion.[21,79,80] The main advantages of estradiol vaginal tablets over other vaginal estradiol platforms are represented in its efficacy in the local alleviation of symptoms, ease of use, a more hygienic administration, increased patient acceptability and a low estradiol loading-dose, which leads decreased systemic adverse events.[9,60,80,108] Yuvafem® is a generic 10 μg estradiol vaginal tablet manufactured by Amneal Pharmaceuticals (Bridgewater, NJ).[109]

A crucial concern regarding the delivery of estrogen through locally administered dosage forms is the amount that enters systemic circulation, which is clinically important with regards to probable adverse events, such as breast cancer and venous thromboembolism.[22,64] Several pharmacokinetic studies showed that local vaginal estradiol formulations are associated with lower systemic exposure compared to oral formulations, implying that the probability of adverse events incidence is also minimum.[4,71] The analysis and determination of estrogen levels are also more complicated in postmenopausal than premenopausal women, due to the reduced sensitivity of many assays to detect low estrogen levels. Several methods are in use for the determination of estradiol including gas chromatography-mass spectrometry, radio-immunoassays, and ultra-sensitive bioassay.

Two studies investigated and compared the systemic absorption of 17β-estradiol from 10 and 25 μg Vagifem® tablets in postmenopausal women.[60,61] Both of these studies showed low systemic absorption (within normal postmenopausal range) for both doses, with higher absorption associated with the 25 μg dose, confirming that the extent of the absorption was dose dependent. Interestingly, the absorption patterns in the one study[61] initially showed significant estradiol absorption at both doses, which decreased significantly by day fourteen, while Notelovitz et al.[60] reported a steady absorption pattern at the beginning and end of the 12-week study period. Both studies used a radioimmunoassay technique to detect systemic estradiol. The number of participants and the study period were 58 women and 12 weeks, respectively, for the study undertaken by Notelovitz et al. with serum estradiol <20 pg/ml as the enrolment criterion, compared to 24 participants, a 2-week study period, and no specification for the baseline serum estradiol levels in the other study.[61] In contrast, a study by Labrie et al.[81] used a sensitive and accurate mass spectrometry assay (chromatography/mass spectrometry), evaluating the systematic bioavailability of estradiol and estrone during the 24 hours following a one week application of 25 μg Vagifem® and 0.625 mg Premarin® cream. Although the study had a small number of participants (10 patients in each group), the findings demonstrated that serum estradiol was increased five-fold during the first 24 hours period after administration of 25 μg Vagifem® or 1 g Premarin®, and that serum estrone increased 150% with Vagifem® and 500% with Premarin® cream after daily administration. These results indicate that systemic effects are likely to occur following administration of these dosage forms.

GSM is also characterized by the incidence of numerous events, such as increased parabasal cells, decreased superficial cells, vaginal epithelium thinning, and an increased pH to more than 5,[6] thus, improvement in these factors and other vaginal symptoms could be

Table 2
Summary of the studies that compared test estradiol vaginal formulations versus placebos, or to other vaginal estrogen formulations.

Study	Treatment	Comparator	Duration of treatment	Design of the study	Objective	Location and No of centers
Rioux et al.[77]	25 μg Vagifem (n = 80) Withdrawal (8)	1.25 mg Premarin (n = 79) Withdrawal (25)	24 weeks	Multicenter, open-label, randomized, parallel-group study	Compare the efficacy safety and acceptability	Canada (6 centers)
Ekin et al.[78]	25 μg Vagifem (n = 24) Withdrawal (3)	5 mg hyaluronic acid tablet (n = 24) Withdrawal (3)	8 weeks	Randomized, controlled study	Compare the efficacy	Turkey (1 center)
Dugal et al.[79]	25 μg Vagifem (n = 48) Withdrawal (6)	0.5 mg estriol vagitories (n = 48) Withdrawal (5)	24 weeks	Randomized, parallel-group, single blind, multicenter trial	Compare the acceptability, efficacy and safety	NA
Ilhan et al.[4]	10 μg Vagifem (n = 30)	2 g Cicatridina ovule (n = 31) And 10 mg Colpotrophine ovule (n = 30)	12 weeks	Prospective and open-label study	Compare the efficacy	Turkey (centers=NA)
Hosseinzadeh et al.[80]	25 μg Vagifem (n = 80) Withdrawal (NA)	Vaginal estrogen cream (n = 80) Withdrawal (NA)	12 weeks	Simple randomized clinical trial	Compare the efficacy, acceptability, and safety	Iran (1 center)
Labrie et al.[81]	25 μg Vagifem (n = 10) Withdrawal (NA)	0.625 mg Premarin (n = 10) Withdrawal (NA)	1 week	A prospective, randomized trial	Evaluation of the systemic bioavailability and the pharmacokinetics	NA NA
Nilsson and Heimer[61]	10 μg Vagifem	24 participants* 25 μg Vagifem	2 weeks	Randomized, double-blind, cross-over study	Determination of the systemic absorption	NA
Notelovitz et al.[60]	10 μg Vagifem (n = 30) Withdrawal (7)	25 μg Vagifem (n = 28) Withdrawal (9)	12 weeks	Double-masked, randomized, parallel group study	Determination of the systemic absorption	USA (1 center)
NCT01779947[82]	Generic 10 μg estradiol tablet	10 μg Vagifem Placebo	2 weeks	An investigator-blind, randomized, parallel-group, placebo-controlled, multicenter study	Compare the safety and efficacy	USA (25 Centers)
Minkin et al.[63]	10 μg Vagifem tablet	79 Participants* Estring ring Estrace cream Premarin cream	5 weeks	Online survey of postmenopausal local estrogen therapy users	Study of patient satisfaction with estradiol vaginal tablets in postmenopausal women previously treated with another local estrogen therapy	USA NA
Eriksen and Rasmussen[83]	25 μg Vagifem (n = 81) Withdrawal (6)	Placebo (n = 83) Withdrawal (4)	12 weeks	Double-blind randomized placebo controlled study	Compare the efficacy	Denmark (centers=NA)
Diem et al.[84]	10 μg Vagifem + placebo vaginal gel (n = 102) Withdrawal (NA)	Placebo vaginal tablet + vaginal moisturizer (n = 100) Withdrawal (NA) Placebo tablet + placebo gel (n = 100) Withdrawal (NA)	12 weeks	A randomized, double-blind, placebo-controlled trial	Compare the effects of a Vagifem tablet and a vaginal moisturizer to placebo on menopause-related quality of life and mood	USA (2 centers)

(*continued on next page*)

Table 2 (*Continued*)

Study	Treatment	Comparator	Duration of treatment	Design of the study	Objective	Location and No of centers
Mashingaidze[85]	4 μg TX-004HR (n = 18) 10 μg TX-004HR (n = 19) 25 μg TX-004HR (n = 18)	Placebo (n = 17) Withdrawal (1)	12 weeks	Multicenter, double-blind, placebo-controlled, phase 3	To evaluate the pharmacokinetics parameters	United States Canada (11 centers)
Pickar et al. [32]	10 μg TX-004HR (n = 24) Withdrawal (0)	Placebo (n = 26) Withdrawal (2)	2 weeks	Single-center, double-blind, placebo controlled phase 2 pilot trial	To evaluate the safety and efficacy	NA (1 center)
Constantine et al.[70]	4 μg TX-004HR (n = 191) Withdrawal (11) 10 μg TX-004HR (n = 191) Withdrawal (14) 25 μg TX-004HR (n = 190) Withdrawal (9)	Placebo (n = 192) Withdrawal (10)	12 weeks	Multicenter randomized, double-blind, placebo-controlled, phase 3 study	Safety and efficacy	USA Canada (89 centers)
Simon et al.[86]	4 μg TX-004HR (n = 191) Withdrawal (NA) 10 μg TX-004HR (n = 191) Withdrawal (NA) 25 μg TX-004HR (n = 190) Withdrawal (NA)	Placebo (n = 192) Withdrawal (NA)	12 weeks	Post hoc analyses for data obtained from a multicenter, double-blind, randomized, placebo-controlled, phase 3 trial	To evaluate improvement of dyspareunia and associated vaginal dryness with a TX-004HR softgel vaginal insert	USA Canada (Centers=NA)
Mirkin et al.[87]	4 μg TX-004HR (n = 22) Withdrawal (NA) 10 μg TX-004HR (n = 25) Withdrawal (NA)	Placebo (n = 25) Withdrawal (NA)	12 weeks	Post hoc analysis for data obtained from a phase 3, prospective, randomized, double-blind, placebo-controlled, multicenter study	To evaluate endometrial progesterone receptor expression in menopausal women	USA Canada (89 Centers)
Casper et al.[88]	Estring (n = 174) Withdrawal (31)	Estriol vaginal pessaries (n = 72) Withdrawal (17)	12 weeks	Randomized open-label comparative trial with parallel groups	Compare the efficacy, safety and acceptability	Austria Switzerland Germany (14 centers)
Barentsen et al.[89]	Estring (n = 83) Withdrawal (11)	Estriol vaginal cream (n = 82) Withdrawal (16)	12 weeks	Open-label, change-over, randomized parallel group trial	Compare the efficacy and evaluation of treatment preference	Netherland (12 centers)
Weisberg et al.[90]	Estring (n = 126) Withdrawal (32)	25 μg Vagifem (n = 59) Withdrawal (7)	48 weeks	Open-label, randomized, parallel-group study	Endometrial safety	Australia (4 centers)
Henriksson et al.[91]	Estring (n = 112) Withdrawal (11)	0.5 mg estriol vaginal pessary (n = 53) Withdrawal (8)	12 weeks	An open, randomized, parallel-group, comparative trial	Efficacy, safety, and acceptability	Sweden Finland Denmark (9 centers)
Ayton et al.[92]	Estring (n = 131) Withdrawal (11)	0.625 mg Premarin (n = 63) Withdrawal (7)	12 weeks	An open, randomised, parallel, comparative multicentre trial	To compare the safety, efficacy and acceptability	Australia (3 centers)
Casper et al.[88]	Estring (n = 43) Withdrawal (10)	Placebo (n = 41) Withdrawal (7)	24 weeks	Double-blinded randomized placebo-controlled in parallel groups	Compare the efficacy	Germany (10 centers)

(*continued on next page*)

Table 2 (*Continued*)

Study	Treatment	Comparator	Duration of treatment	Design of the study	Objective	Location and No of centers
Eriksen[93]	Estring ($n = 53$) Withdrawal (17)	No treatment (control group) ($n = 55$) Withdrawal (6)	36 weeks	A multicenter, randomized, open, parallel-group study with an untreated control group	To detect the preventive effect of the Estring) on recurrent urinary tract infections in postmenopausal women	Norway (15 centers)
Smith et al.[94]	2 mg estradiol vaginal ring ($n = 222$) Withdrawal (56)	NA	54 weeks	The study was designed as a multiple independent trial	To study the efficacy, safety and acceptability	Sweden (7 centres)
Speroff Group[95]	50 μg Femring ($n = 113$) Withdrawal (14) And 100 μg Femring ($n = 112$) Withdrawal (11)	Placebo ($n = 108$) Withdrawal (29)	13 weeks	Double-blind, randomized, placebo-controlled trial	To assess the efficacy, tolerability, and acceptance for vasomotor and GSM symptoms	USA (35 centers)
Buckler et al.[96]	50 μg Menoring + placebo tablet ($n = 84$)	1mg oral estradiol tablets (Elleste Solo) + placebo ring ($n = 75$)	24 weeks	Prospective, multicentre, randomized, double-blind, comparator-controlled, parallel group study	Evaluate the efficacy and acceptability	United Kingdom (21 centers)
Antoniou et al.[97]	50 μg vaginal ring + progesterone suppository ($n = 28$)	50 μg estradiol patch + levonorgestrel IUD ($n = 28$)	12 months	NA	To compare the efficacy, safety and acceptability	Greece (1 center)
Hamada et al.[98]	Vaginal ring deliver in vitro estradiol 160 μg/day and progesterone 20 mg/day ($n = 8$) Withdrawal (1) Vaginal ring deliver in vitro estradiol 160 μg/day and progesterone 10 mg/day ($n = 12$) Withdrawal (2)	NA	16 weeks	NA	To investigate whether vaginal rings delivering estradiol and progesterone could prevent endometrial hyperplasia and relieve climacteric symptoms	NA
Martin et al.[99]	Estrace vaginal cream ($n = 20$) Withdrawal (0)	1.25 mg Premarin (n = 10) Withdrawal (1)	2 weeks	NA	To evaluate systemic absorption and sustained effects of two estrogen vaginal cream preparations	NA NA
NCT03294538[100]	Generic 0.01% estradiol cream ($n = 268$) Withdrawal (5)	0.01% Estrace cream ($n = 262$) Withdrawal (5) Placebo ($n = 133$) Withdrawal (2)	9 days	A randomized, double-blind, placebo-controlled, parallel-design, multiple-site study	To evaluate the therapeutic equivalence and safety	USA (44 centers)
Archer et al.[6]	0.003% estradiol vaginal cream ($n = 287$) Withdrawal (22)	Placebo cream ($n = 289$) Withdrawal (28)	12 weeks	Phase 3, randomized, double-blind, placebo controlled, multicenter study	To examine the efficacy and safety	NA

(*continued on next page*)

Table 2 (*Continued*)

Study	Treatment	Comparator	Duration of treatment	Design of the study	Objective	Location and No of centers
Kroll et al.[101]	0.003% estradiol vaginal cream (n = 277) Withdrawal (22)	Placebo cream (n = 273) Withdrawal (31)	12 weeks	Phase 3, randomized, double-blind, placebo controlled, multicenter study	To compare the safety and efficacy	NA
Tanmahasamut et al.[59]	25 μg estradiol gel (n = 40) Withdrawal (2)	Placebo (K-Y® Jelly) (n = 40) Withdrawal (3)	8 weeks	A randomized double-blind controlled trial	To evaluate the efficacy and safety	Thailand (1 center)

* Participants randomized between the treatment and the comparator.
NA: Not available.

used as indicators for system effectiveness in treating the symptoms of GSM.[77,110] The efficacy of estradiol vaginal tablets were evaluated and compared to other estrogen-containing dosage forms in numerous studies, with an improvement in subjective and objective signs and symptoms, without major safety concerns demonstrated.[4,77-80,90] A study undertaken by Dugal et al.[79] followed 96 postmenopausal women for 24 weeks to evaluate and compare the efficacy of 25 μg Vagifem® tablets to estriol vagitories. The results showed that the efficacy of both treatments is equivalent. However, the endometrial thickness was increased more in the Vagifem® group during the initial two weeks, but the baseline thickness was restored when the dosing schedule reduced to two applications per week. In another study by Hosseinzadeh et al.,[80] 160 postmenopausal women were randomized into two groups for treatment with Vagifem® or with a vaginal estrogen cream for 12 weeks. Both treatments significantly improved symptoms of atrophic vaginitis, without significant difference between the two groups. Similarly, Rioux et al.[77] conducted a multicenter, open-label, randomized, parallel group study on 159 menopausal women to compare the efficacy of Vagifem® and a vaginal estrogen cream. The authors concluded that the efficacy of both the tablet and cream was equivalent in relieving symptoms of atrophic vaginitis. In addition, Vagifem® demonstrated localized effects with no significant increase in systemic estradiol levels or estrogenic side effects.

With the increased prevalence of GSM, there is also a need for cheap therapeutic options, especially in developing countries, where combined oral contraceptive tablets are less expensive compared to vaginally-administered estrogenic-medications.[111] Chompootaweep et al.[111] compared the efficacy of easily obtainable and less expensive combined oral contraceptive pills (250 μg levonorgestrel and 30 μg ethinyl estradiol) with conjugated equine estrogen cream for the treatment of urogenital symptoms in 40 postmenopausal Thai women. Twenty participants applied one tablet every week vaginally for 8 weeks, while the other 20 participants applied the cream in a decreasing dosage of 1 gram three times per week to 1 gram once per week by Week 3. All tablet users had a marked improvement in vaginal dryness, dyspareunia, urinary urgency, and urinary frequency, with no statistically significant differences between the groups. Also, in both groups, pH decreased and maturation and karyopyknotic indexes were improved compared to baseline.

Safety of Vaginal Tablets

With the vaginal administration of estrogens offering reduced systemic absorption compared to orally delivered estrogens, the occurrence of serious side effects are expected to be minimal.[68,87] The safety of vaginally estradiol dosages are mainly screened through the occurrence of adverse events, laboratory markers findings, and endometrial biopsies using ultrasonography.[77] The most common adverse events associated with vaginal estrogens administration are vaginal bleeding, candidiasis and discharge, in addition to the risks associated with systemic estrogen exposure, which may lead to venous thrombosis and endometrial hyperplasia.[5] The risk of endometrial proliferation associated with the use of unopposed estrogen furthermore remains a crucial concern. The serum estradiol levels which can lead to endometrial proliferation are approximate more than 70 pmol/liter, which is the higher limit of the normal postmenopausal range, with some inter-individual difference.[90] Several studies demonstrated that transdermal and oral estrogen treatment results in 1-28% and 0.2-3% of all endometrial hyperplasia and neoplasia cases, respectively, while locally applied estrogen causes 0-2% of all cases. Thus, no categorical association between vaginal estrogen and endometrial hyperplasia has been established.[112]

However, the lack of evidence on long term estrogenic adverse events makes the systemic exposure from vaginal delivery of great concern and may be the foundation to contraindicate vaginal

formulations for some postmenopausal women.[113] In a study to evaluate the endometrial safety of 10 μg estradiol vaginal tablets administered for 52-weeks, Simon et al.[114] evaluated the incidence rate of endometrial hyperplasia and carcinoma in two joint studies with 541 total participants. The biopsy data revealed that the incidence rate of hyperplasia and carcinoma is only 0.52% per year from 386 evaluable samples, and thus indicated there is no increased risk of endometrial hyperplasia and carcinoma in postmenopausal women using 10 μg estradiol vaginal tablets for 1 year under the same study conditions. The impact of the Vagifem® dosage regimen on the endometrial tissues was further evaluated by Mettler and Olsen.[115] During the first two weeks, all participants with GSM were treated with one 25 μg Vagifem® tablet daily. Thereafter the participants were randomized in an open controlled, two parallel groups, receiving either Vagifem® two times per week (34 participants), or once weekly (17 participants), for 50 weeks. After one year of treatment, no endometrial hyperplasia was observed in 45 patients who completed the one-year study period. Nine of the patients (two of the once weekly and seven of the twice weekly maintenance therapy groups) continued treatment for a further 12 months, with the application of one tablet twice per week. From this group, one patient of the once weekly and two patients of the two weekly maintenance therapy groups showed weak endometrium proliferation. All nine patients who completed two years of treatment showed atrophic endometrium. These findings indicate that the twice weekly administration of 25 μg Vagifem® is the lowest safe and effective dose for the long-term treatment of urogenital symptoms in postmenopausal women.

The guidelines of the North American Menopause Society and the International Society for the Study of Women's Sexual Health recommend the use of non-hormonal therapies as the treatment of choice for women at high risk of breast cancer and low dose vaginal formulations as second-line for women whose symptoms persist with non-hormonal therapies.[10] Recently, breast cancer survivors increasingly use aromatase inhibitors more than tamoxifen, leading to an increased induction of GSM symptoms.[116] To study the concomitant use of vaginal estrogen with aromatase inhibitors, Kendall et al.[116] analyzed the serum levels of estradiol in seven women, where six of them used 25 μg Vagifem® tablets and one used Premarin® cream. The serum estradiol was found to be $\leq$5 pmol/l at baseline and increased to an average of 72 pmol/l at Week 2, then decreasing to less than 35 pmol/l in most participants, although significant further increases were seen in two participants. The authors found that the 25 μg Vagifem® tablet significantly increased systemic estradiol exposure and stated that it is contraindicated in women with breast cancer undergoing aromatase inhibitors treatment, unless there is ability for regular screening for serum estradiol. This study, however, included a small number of participants and used a radioimmunoassay to determine estradiol levels.

Acceptability

Patient acceptability is a vital feature in GSM treatment as therapy is mostly often for an extended period.[79] Additionally, the selection of vaginal estrogen formulations is highly based on the user's preferences and needs, due to most formulations having been demonstrated to be effective with a high safety profile.[63] Many studies have shown higher acceptance rates for estradiol tablets compared to other vaginal delivery systems and placebo formulations.[79,80,84,117] This high acceptance rate was primarily attributed to the minimal or absence of leakage and messiness, low pain during application, ease of use and high hygienic value, in addition to a higher improvement of mood and quality of life compared to a placebo. From a design and formulation perspective, advantages are attributed to the small tablet size, tablet muco-adhesion, and deep positioning during administration. In a study conducted by Minkin et al.[63], an online survey was used to study the reason for changing to vaginal estradiol tablets

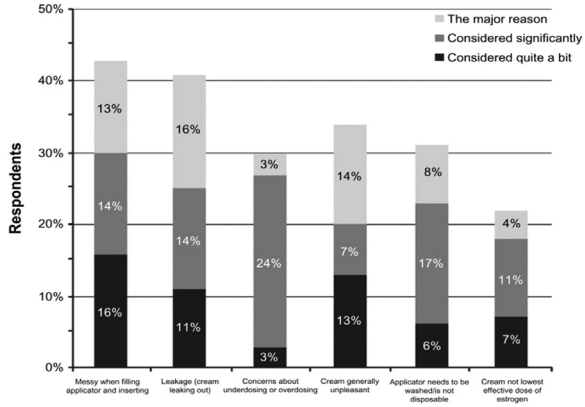

Figure 3. Reasons for switching from a vaginal cream to a vaginal tablet. Reproduced from Minkin et al.[63], © 2013 Minkin et al, publisher and licensee Dove Medical Press Ltd.

from other estradiol preparations such as creams, rings, and conjugated estrogen creams. The respondents demonstrated that the tablets are preferable to the ring or cream. The factors for shifting from vaginal cream to vaginal tablet are depicted in Fig. 3. Efficacy, ease of use, and longer adherence to treatment were the main reasons behind the preference of tablets. However, in addition to the small number of participants included in this study (79 participants), only 9% had previously used an estradiol ring, which may reduce the evidence of tablet preference over the vaginal ring. Furthermore, in clinical practice settings, patients using vaginal tablets demonstrated a significantly longer adherence to treatment than those using vaginal creams ($p < 0.01$).[118]

Little data and information exist on the efficacy, safety and acceptance of the generic 10 μg estradiol vaginal tablets, however, two phase 3 clinical studies (NCT01779947 and NCT02668796) compared the safety and efficacy of two generic 10 μg estradiol vaginal tablets to the FDA approved 10 μg Vagifem® tablet in postmenopausal women with moderate to severe vaginal atrophy.[82,119] The data from these trials are however not available.

Softgel Capsules

TX-004HR, commercially traded as Imvexxy® (TherapeuticsMD, Inc., Boca Raton, FL), is 17β-estradiol low dose softgel capsule which adheres to the vaginal epithelium after manual insertion to provide a rapid improvement in vulvovaginal atrophy symptoms in postmenopausal women with negligible to deficient systemic absorption.[52,110,120,121] In 2018, the FDA approved two dosages (4 μg and 10 μg) of TX-004HR for the treatment of moderate to severe dyspareunia.[54,87,110,122] TX-004HR products are tear-shaped, small softgel capsules with about 1.58 cm length, encased by gelatin as the capsule shell.[71,110] The dosing schedule of TX-004HR includes the application of one capsule daily for the first two weeks, followed by one capsule twice per week.[52,68,71] It is recommended to commence the treatment regimen with the lowest 4 μg capsule dosage.[68,71]

The main advantages of TX-004HR capsules lies in its ability to afford local efficacy, with low systemic absorption, ease of administration, avoidance of messiness, and convenience.[123] The TX-004HR products contrasting to Vagifem®, are contraindicated in the case of suspected breast cancer, active arterial or history of venous thromboembolism, idiopathic vaginal bleeding, and estrogen-dependent neoplasia.[68]

The TX-004HR softgel capsule was designed to offer local estrogenic effect without escalating the systemic absorption of estradiol.[123] The position at which the dosage form is inserted in the vaginal cavity could also have an influence in the degree of systemic

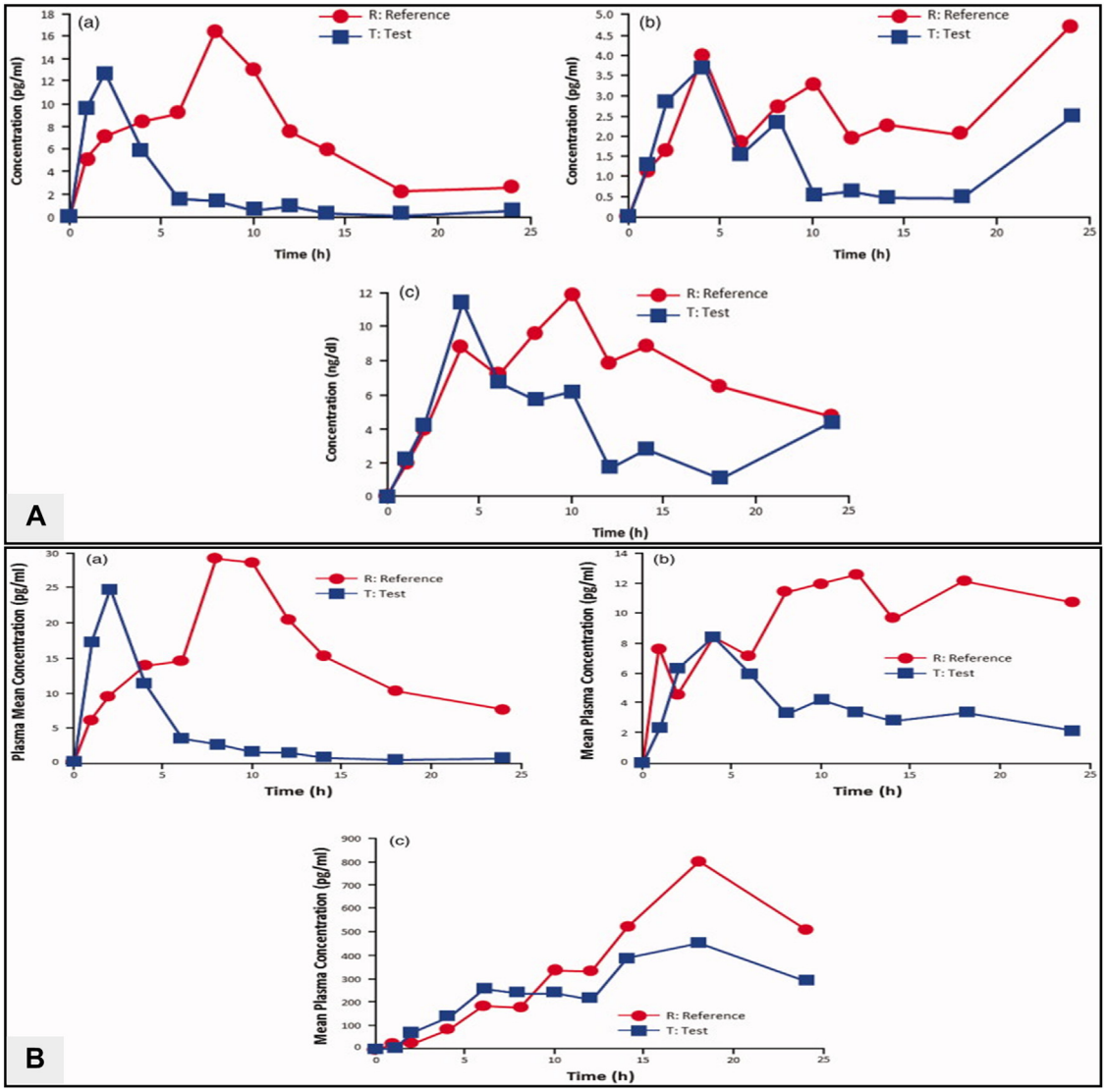

Figure 4. Linear plot of baseline-corrected mean plasma concentration versus time for (a) estradiol, (b) estrone, and (c) estrone sulfate after treatment with the test and reference preparations, each at (A) 10 μg and (B) 25 μg. Test product: TX-004HR; TherapeuticsMD, Inc., Boca Raton, FL. Reference product: Vagifem®; Novo Nordisk, Plainsboro, NJ. Reproduced from Pickar et al.[123] © 2016 J. H. Pickar.

estradiol absorption from vaginal formulations. The lower one third of the vaginal cavity absorbs estradiol less systemically than the upper one third, with the vulva less absorptive than the vaginal epithelium.[5] Also, the delivered dose could affect the amount of estradiol that reaches the systemic circulation. The low dose estradiol (4, 7.5, and 10μg) platforms result in the lowest systemic absorption, while the intermediate dose (25 μg) provides to some extent higher systemic exposure.[22,124] Additionally, other factors influencing systemic absorption include the carrier used to deliver the estradiol, and the type of delivery system.[22,61,62] In a 12-week sub-study[85] of the REJOICE trial, the pharmacokinetics parameters of 4, 10, and 25 μg TX-004HR softgel capsules were assessed compared to a placebo. The findings showed that the softgel capsules exhibited minor to very low systemic absorption. Additionally, there were no significant differences in estradiol measures of AUC, C_{max}, C_{avg}, C_{min}, and T_{max} between the 4 μg softgel capsules dose and the placebo. The 10 μg dose showed C_{max} was significantly higher than the placebo on Day 1 with no difference on Day 14. The pharmacokinetic parameters of the 25 μg softgel capsules dose were also significantly higher than the placebo, but the amount of absorbed estradiol remained within the normal range for postmenopausal women.

Pickar et al.[123] assessed the pharmacokinetics and safety of the 10 and 25 μg doses of TX-004HR softgel capsules (applied to lower part of the vagina) compared to the corresponding Vagifem® tablet doses (inserted deeply in the vagina) in two randomized, single-dose, two-way cross-over, relative bioavailability trials in postmenopausal women. In both studies, the degree of systemic estradiol, estrone, and estrone sulfate exposure with softgel capsules were significantly lower than that of Vagifem®. Also, both doses of softgel capsules exhibited a faster systemic absorption pattern and a rapid return to baseline serum estradiol levels compared to Vagifem® (Fig. 4). This faster absorption pattern probably reflects a more rapid release of estradiol from the oil carrier in the softgel capsules than that from the cellulosic base of the Vagifem® tablets.

The TX-004HR softgel capsules at all doses compared to placebo has also been noted to have a strong positive influence on vaginal physiology, resulting in improvement of the subjective and objective symptoms of GSM.[52,121] This improvement in symptoms is dose-dependent and observed as earlier as Week 2 of starting the treatment, compared to Week 12 reported with conjugated estrogens.[121] Two 12-week studies also evaluated the efficacy of 4, 10, and 25 μg doses of TX-004HR softgel capsules in treating severe to moderate

dyspareunia. All three doses of TX-004HR significantly improved dyspareunia and vaginal dryness compared to the placebo.[70,86]

Safety of Softgel Capsules

The topical vaginal administration of estrogens has been shown to have a lower systemic absorption compared to oral administration. Thus, vaginal administration of estrogen is expected to exhibit lower adverse events. Nonetheless, all FDA-approved local estrogen dosages prescribing information enclose a black-box warning concerning the risk of endometrial and breast cancer, cardiovascular disorders, and probable dementia.[37] To assess the safety of 10 μg TX-004HR softgel capsules, Pickar et al. conducted a 2-week double-blind, placebo-controlled, single center study. Results showed that 28% of participants experienced adverse events. However, all side effects were mild, and no deaths or serious events were reported.[32] Another recent study[87] used endometrial progesterone receptor expression as a biomarker for estradiol exposure after administration of 4 and 10 μg softgel capsules. The findings indicated that there is no significant difference in endometrial progesterone receptor expression between the baseline and Week 12. This indicates that both doses are not expected to pose a potential endometrial safety concern.[87] It is however noted that both studies had a relatively small sample size, with none directly measuring endometrial thickness. Additionally, neither included a safety evaluation of TX-004HR at a 25 μg dose and had short study times.

Acceptability

TX-004HR was designed with some properties to increase its acceptance by users, such as features to be used without an applicator, designed to be less messy and the dose could be applied at any time of the day to start the treatment.[121] A survey of five questions was employed to assess the acceptability of TX- 004HR at the three strengths. Most of the participants rated the ease of product insertion as good to excellent (75% to 82.6%), and about 90% of participants found it was easy to use. The majority of participants rated the product as very satisfied or satisfied, compared to the placebo. Also, most patients very much or somewhat preferred TX- 004HR over other treatments they had used previously (Fig. 5) and would probably or definitely use TX- 004HR again. The satisfaction, ease of insertion, and possibly reuse of TX-004HR were correlated with improved dyspareunia, vaginal pH, and vaginal dryness.[120] The increased patient acceptance and satisfaction may therefore lead to a higher adherence to prescribed medication regimens and lower treatment failure rates. This high level of acceptance may be due to several features of softgel capsules such as the ability to administer without an applicator in a more hygienic manner, no need to measure individual doses, less concern about systemic absorption, rapid dissolution after application, and the muco-adhesive properties of the capsules, which can decrease the rate of messiness. However, one of the limitations of this study is the need for a direct comparison with other intravaginal estradiol delivery systems.

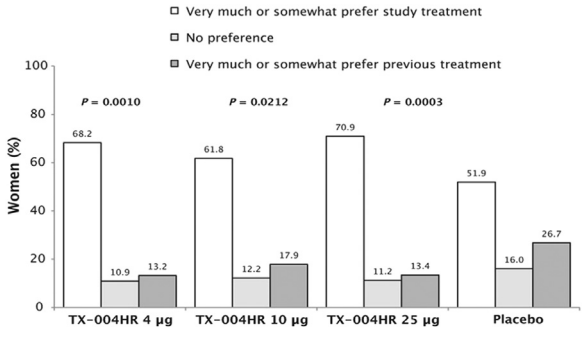

Figure 5. Treatment preference over previously used vulvar and vaginal atrophy treatments. *P* value versus placebo. Reproduced from Kingsberg et al.[120], © 2017 The Author(s).

Vaginal Rings

The vaginal ring is a drug delivery system affording the controlled release of therapeutic agents for localized or systemic effects over an extended period of time.[125] Vaginal rings can also be utilized for contraception and for the relieving of GSM symptoms.[90,125] The intravaginal ring, in addition to its prolonged duration of action, can overcome many of shortfalls associated with other vaginal delivery systems such as the inadequate retention time inside the vaginal cavity and difficulty of use, thus raising treatment effectiveness and patient acceptance.[95,126] Vaginal rings, however, have some disadvantages, such as an increased vaginal discharge and the possibility of expulsion.[74]

US FDA approval has been granted for the use of estradiol vaginal rings in the treatment of GSM and offers additional options for this condition.[127] Several 17β-estradiol vaginal rings are currently in commercial use, including a ring which minimally increases the level of estradiol in plasma, however not more than the normal menopausal range.[128] The second ring increases systemic estradiol to 40.6 pg/mL and 76 pg/mL at doses of 50 μg/day and 100 μg/day, respectively, with this ring used to treat both GSM and the systemic symptoms of menopause. Other rings for combination hormonal therapy have been described in the literature but are not commercially available.[128]

One of the approved products is the Estring® vaginal ring. Estring® is a soft, flexible device with an outer 55 mm diameter and 9 mm thickness, comprised of an inner core containing 2 mg of 17β-estradiol as a reservoir and covered with a release rate controlling membrane made of a silicone elastomer to release about 7.5 μg of 17β-estradiol per day for a period of 90 days.[88,93,127,129,130] This consistent release provides mean steady plasma concentrations of 5.7 and 7.6 pg/mL, which is suitable for the treatment of GSM.[95]

Due to its relatively high polarity, 17β-estradiol cannot be clinically delivered at a rate of at least 50 μg per day from a hydrophobic vaginal ring with a reservoir design. Therefore, the use of a suitable estradiol ester with a sufficient balance of hydrophobic and aqueous property, such as estradiol acetate, may enable effective sustained delivery at suitable rates for the treatment of the vasomotor symptoms of menopause.[13] Estradiol-3-acetate has improved solubility in ring-forming polymers and is rapidly hydrolyzed to active estradiol in plasma and tissues. These properties enable it to provide release rates of 100 or 50 μg per day for 90 days, offering average serum concentrations of about 280 and 150 pmol/L, respectively. These serum concentrations resemble that achieved by estradiol transdermal patches and is adequate to control the vasomotor symptoms of menopause.[96,131]

A novel estradiol acetate vaginal ring of a reservoir system was approved for use in the UK in 2001 and marketed as Menoring®. In 2002, it was approved by the US FDA under the name of Femring®.[128,132] This novel vaginal ring is approved for the alleviation of both vulvovaginal atrophy and the vasomotor symptoms of menopause.[10,21,95] Femring® is a flexible, soft, off-white ring, composed of estradiol acetate embedded in a central core shielded with a release-rate controlling membrane. The amount of estradiol acetate load is either 12.4 mg or 24.8 mg to consistently release 50 or 100 μg of estradiol acetate per day, respectively, for a period of 90 days.[133] The released estradiol acetate is rapidly hydrolyzed and converted to naturally occurring estradiol. The release of 50 or 100 μg estradiol per day results in average daily plasma concentrations of 40-76 pg/mL[22,95] Due to this systemic absorption of estradiol, women with an intact uterus need to use concomitant progestogen therapy to oppose

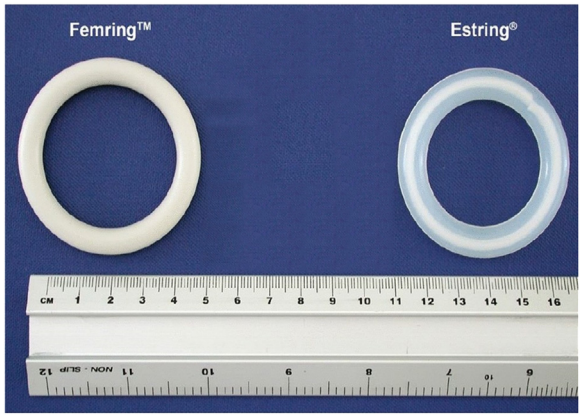

Figure 6. Images of the FDA approved vaginal rings for hormone replacement therapy (Estring® and Femring®) adapted from Zhao et al.[134] © The Author(s) 2022.

the estradiol effect and avoid endometrial stimulation.[10,22] Fig. 6 illustrates the diameter and cross-sectional diameter of Femring® and Estring®.

Akcess Medical Products Inc. of King of Prussia, PA, prepared rings comprising of a core of 4% 17β-estradiol in a dimethylsiloxane/vinylmethylsiloxane matrix. These rings have an *in vitro* release rate of 100, 150, 200 μg/day according to the breadth of the outside silicone membrane: 0.47, 0.28, and 0.21 mm, respectively. The dipping process was utilized in order to coat the rings with the outside release rate controlling membrane.[135] Also, FEI Technologies (Plainsboro, NJ) have developed two models of toroidal shape vaginal rings with an inner core containing 4% of 17β-estradiol. One version was designed to release average of about 140 μg per day *in vitro* with the other designed to release about 60 μg per day. Both models are approximately 56 mm in overall diameter and 8 mm in cross-sectional diameter.[136] Other rings composed of a toroidal silicone elastomer tubing loaded with estradiol and low or high dose progesterone include the rings manufactured by FEI Technologies, designed with a 55 mm diameter and a 9 mm cross-sectional diameter. Both versions contain 0.36 g of 17β-estradiol, and 3.6 g or 1.8 g of progesterone, respectively. The low-dose and high-dose rings have *in vitro* drug release patterns of 7, 5, and 4 mg, and 13, 8, and 6.5 mg of progesterone per day, at 30, 90, and 180 days, respectively. Corresponding to that, both rings release estradiol at rates of 160 μg, 150 μg, and 140 μg per day. These rings therefore provide a promising method for hormone replacement therapy with effective endometrial proliferation prevention.[137]

The development of ethylene vinyl acetate-based vaginal rings that release a combination of estradiol and progesterone may also provide additional therapeutic options to those currently available. Novel ethylene vinyl acetate vaginal rings that can deliver a combination of estradiol at a rate of 160 μg and progesterone at 4 μg or 8 μg per day, were prepared by employing ethylene vinyl acetate fibers of various length and drug loading.[138] The administration of these estradiol vaginal rings by insertion into the upper third of the posterior vault of the vagina only once every three months for a maximum uninterrupted treatment period of 24 months,[19,95,127] in addition to a rare expulsion rate, might lead to increased patient acceptability.[125]

One of the most vital issues during the designing and development of vaginal rings is the selection of the appropriate polymers, which have a significant influence on drug release patterns. The solubility and diffusivity of the drug in the polymer, and the polymer's biocompatibility and stability, are the most important factors that should be considered. The intravaginal rings are made mainly from three polymers, silicone, polyethylene-co-vinyl acetate, and polyurethane.[74] The most common silicone is polydimethylsiloxane which is formed by the attachment of methyl groups to the siloxane backbone and is employed extensively in drug delivery and medical devices due to its biocompatibility and flexibility[74]; also, polydimethylsiloxane, is recognized by its hydrophobicity and permeability to diffuse of different substances.[139] In addition, the polydimethylsiloxane matrices are characterized by their ability to release steroids in a steady rate fashion for an extended period of time.[140] Two common silicone elastomers are used to manufacture estradiol vaginal rings, additive-cured and condensed-cured type silicone elastomers. Additive-cured silicone elastomers are also used in the formulation of Estring®, while Femring® is manufactured using condensed-cured silicone elastomers.[141] Vaginal rings were also initially developed as homogenous systems with a steroidal drug evenly distributed in a polysiloxane matrix. This resulted in a high immediate burst release, and to overcome that shortfall, the rings were coated with a drug-free release-controlling membrane.[128]

In a study by Woolfson et al.,[13] reservoir intravaginal rings, loaded with 17β-estradiol or its acetate ester were prepared by employing an injection molding method. A similar method was described by Malcolm et al.[142] to prepare matrix-type vaginal rings loaded with 17β-estradiol or estradiol acetate, except by coating the rings with a release rate-controlling membrane. Also, Weiss et al. reported a method for preparation of a novel ethylene vinyl acetate vaginal ring loaded with 17β-estradiol and progesterone.[138]

Generally, all the investigational or commercially available estradiol vaginal rings are either a reservoir system, in which there is a release rate controlling membrane surround the drug core, or of a matrix system, in which the estradiol is dissolved or dispersed in a polymeric carrier. In the reservoir type, the estradiol diffuses through the membrane due to concentration gradient and the release rate usually remains constant over time. Interestingly, the release of estradiol from one reservoir type ring designed to release 160 μg/day demonstrated initial burst release during the first 4 days, followed by a steady release to 100 days.[136]

During pharmaceutical product development, it is important to understand the relation between the *in vitro* and *in vivo* release which allows for the prediction of *in vivo* performance. A study by Nash et al.[135] determined the relation between the *in vitro* release of three ring releasing 200, 150, and 200 μg/day and the *in vivo* release in 21 postmenopausal participants over 22 days. The authors reported that all three doses showed identical plasma estradiol levels to *in vitro* estradiol release, with plasma estradiol levels sufficient to control menopausal symptoms.

Safety of Vaginal Rings

The uncontrolled continuous release of the estrogens associated with most local vaginal estrogens delivery systems may result in increased estrogen levels in plasma and vaginal tissue. The estradiol vaginal ring was designed to avoid this problem.[143] The Estring® vaginal ring is designed to release about 0.8 mg of estradiol during the first 90 days of insertion, with a systemic absorption of about 8% of the total daily estradiol released. This reduced systemic absorption of the estradiol minimizes the possibility of endometrial proliferation. The initial burst release is also not incremental and the amount of systemic absorption of the estradiol is lower in the insertion of the second and following rings. This effect is most likely due to the vaginal epithelium maturation following the initial ring.[127] In a multicenter, randomized, open-label, parallel-group study carried out by Weisberg et al. to evaluate and compare the systemic absorption of estradiol released from Estring® and Vagifem®, the results showed increased serum levels of estradiol and estrone in both groups throughout the treatment period, but within the normal range of postmenopausal women. The mean levels of serum estradiol in the Estring® and Vagifem® groups were found to be 16±22 pmol/l and 15±33 pmol/l at baseline and increased to 49±64 pmol/l and 36±51 pmol/l at Week 24, respectively, lessening to 20±19 pmol/l in the

Estring® group and approximately steady in the Vagifem® group at Week 48. Similarly, the mean levels of serum estrone in the Estring® group were found to be 1±0.67, 1.4±0.86 and 1.57±0.89 nmol/l, while the Vagifem® group mean serum estrone levels were 1.14± 1.76, 1.39±0.82 and 1.69±1.48 nmol/l, at baseline, Week 24 and Week 48, respectively.[90]

Some vaginal rings have however been constructed to provide systemic levels of estradiol. Femring® intravaginal rings allow accurate, controlled administration of drug for up to 1 year. Woolfson et al. demonstrated that an almost constant level of estradiol could be provided in healthy postmenopausal human volunteers for several months using an intravaginal ring.[13] Another early study reported the preparation of two forms of vaginal rings; one as matrix ring loaded with 400 mg of estradiol and the other as a three-layer ring, in which the middle layer was loaded with 100, 200, or 400 mg estradiol. The ability of the ring to deliver systemic estradiol was examined in 14 oophorectomized and postmenopausal women. Both ring designs resulted in burst release after insertion, with the three layer design returning to base line levels within one day, while the matrix type maintained estradiol levels for a minimum of one week above 300 pg/ml, and thereafter at 109-159 pg/ml for a period of 90 days.[144]

The controlled continuous release vaginal ring delivery systems employed for hormonal replacement therapy have also been shown to be effective for GSM, compared to a placebo and other vaginal estrogen-medications. Casper et al.[88] evaluated the efficacy and safety of Estring® in comparison to a placebo and 0.5 mg estriol pessaries in two trials. The Estring® group showed significant improvement in dyspareunia, maturation value, and reduced pH value compared to the placebo group. On the other hand, even though the participants treated with Estring® exhibited higher treatment rates than the pessaries group, both dosages showed equivalent efficacy at Weeks 3 and 12. Additionally, the occurrence of adverse events was not significantly different between the groups. Few participants reported non-serious adverse effects such as local irritation of the vagina, while the Estring® group showed a minimal increase of endometrial thickness by 0.3 mm. A similar study[91] evaluated and compared the efficacy of Estring® with 0.5 mg estriol pessaries. The subjective and objective symptoms were improved excellently in both treatment groups. The vaginal cytology assessment showed significant vaginal epithelium maturation in the Estring® group, compared to the pessary group. In addition, at Week 12, 20% of the Estring® group demonstrated vaginal mucosa atrophy, compared to 58% of the estriol pessaries group.

A study by Weisberg et al.[90] also compared the efficacy of Estring® versus Vagifem® in the alleviation of GSM. The results demonstrated that the urogenital symptoms were improved greatly in both groups, with the pH of the vagina reduced to 5 at three months, and the signs of atrophy decreased from about 90% to about 15% at Week 48. In addition, 8% of the Vagifem® group had fully mature vaginal epithelium, compared to about 26% of the Estring® group. Another study by Smith et al.[94] evaluated the effect of a vaginal ring containing 2 mg 17β-estradiol in a silicone core (E2-IVR; US Pat 4888074-A and 4,871,543) on the atrophic vaginal mucosa. The hormone dosage was controlled by changing the surface area of the ring. The vaginal atrophy symptoms were improved with a dosage of 5-10 μg/day when used for 90 days without endometrial proliferation.

The effectiveness of vaginal rings that deliver high doses of estradiol have also been evaluated in many studies. These novel vaginal rings produce a constant plasma concentration of estradiol over an extended period of time, sufficient to effectively treat both the vasomotor and genitourinary symptoms of menopause. A study by Nash et al.[136] evaluated vaginal rings delivering 60 and 140 μg per day, *in vitro*, for the relief of menopausal symptoms. The mean estradiol levels were 123 and 307 pmol/l for the 60 and 140 μg estradiol releasing rings, respectively, and the estrone levels were higher than estradiol levels by 2.6 and 1.7-fold for the low and high dose ring respectively. The authors found that the rings were effective in correcting genitourinary symptoms and reducing hot flashes by 80% over 6 months. Another double-blind, randomized, multicenter study evaluated and compared the efficacy and safety of a 50 μg/day Menoring® to a 1 mg per day oral estradiol formulation in 159 postmenopausal women. The Menoring® demonstrated significant reduction in vasomotor symptoms severity and frequency similar to that of oral dosage forms. Moreover, Menoring® showed excellent acceptability and was well tolerated by the participants.[96]

In addition to its effectiveness in controlling GSM, estradiol vaginal rings have shown beneficial effects in controlling urinary tract infections associated with decreased estrogen levels during menopause. Eriksen[93] evaluated the ability of Estring® to prevent the recurrence of urinary tract infections in postmenopausal women. The findings demonstrated that Estring® was valuable in delaying and reducing the recurrence of urinary tract infections per year, with the ability to improve other urogenital symptoms among the postmenopausal women significantly. In addition, Estring® is well tolerated and has exhibited a safe profile.

The risk of endometrial stimulation associated with the long term use of unopposed estrogens therapy is, however, considered a major concern.[90,91] The estradiol release pattern from the vaginal ring is consistent at a level of about 7.5 μg/day after a short period of initial burst release. This release pattern is expected to result in minimized systemic estradiol absorption, resulting in a decreased probability of serious events occurrence. Many studies have evaluated the safety profile of estradiol vaginal rings compared to other estrogens delivery systems. Two relatively short-term studies[91,92] compared the safety of Estring® to 0.625 Premarin® cream and estriol pessaries. The findings showed that there is no significant difference between the ring and cream in the incidence of bleeding or endometrial response following a progestogen challenge test. Additionally, the events reported by the Estring® group were vagina burning sensation, breast enlargement, migraines, edema, and urinary incontinence. On the other hand, the estriol-treated patients reported vaginal itching, breast enlargement and pain, with one patient reporting severe bleeding on Days 2 through 5. Another study in 2005[90] looked at the potential for a local effect on the endometrium with the longer-term use (48-weeks total) of Estring® and Vagifem®. The safety of treatment on the endometrium was evaluated by measuring the endometrial thickness and the progestogen challenge test at baseline and Week 48. The findings demonstrated that there was no significant difference in the change in endometrial thickness between the two groups (p=0.81). After the progestogen challenge test, bleeding was uncommon, although there is a statistically significant difference between the two groups, where no Estring® group participants had bleeding, while four participants of the Vagifem® group reported this event.

In postmenopausal women with an intact uterus, the use of estrogen containing products with high doses, further increases the risk of endometrial stimulation. To reduce this risk, it is recommended to add a progesterone derivative with the aim to oppose the effect of estrogens on the uterus. Maruo et al.[137] studied estradiol vaginal rings loaded with low doses of progesterone for hormonal replacement and endometrial proliferation. The vaginal rings, which delivered estradiol at a rate of about 150 μg/day and progesterone of about 9 mg/day or 5 mg/day, were used continuously for 4 and 6 months by postmenopausal women. The results showed an improvement in vasomotor and vaginal symptoms and a decreased incidence of bleeding relative to the pre-treatment status. Also, the authors concluded that the used doses of progesterone were adequate to prevent endometrial proliferation.

A similar study by Hamada et al.[98] evaluated two models of vaginal rings to relieve menopausal symptoms in postmenopausal

women with an intact uterus and prevent endometrial hyperplasia during a four month study period. The models were designed to release 10 mg and 20 mg of progesterone per day *in vitro*, respectively. Both rings were also designed to release 160 μg estradiol per day *in vitro*. There was a significant reduction in the incidence of hot flashes and night sweats, and a noticeable improvement in mood. Participants in both groups reported an increase in vaginal discharge during the first 6 weeks. The incidence of vaginal bleeding was more common among participants in the high progesterone ring group. Endometrial assessments in both groups showed endometrial thickness of less than 3 mm throughout the treatment period. Hence, the combined estradiol-progesterone vaginal ring could potentially be used for long-term hormone replacement therapy with effective protection of the endometrium.

Acceptability

The acceptance of the vaginal ring remains of high interest as the ring may dislodge and also requires a health care practitioner to insert a new ring every 90 days. A systemic review[125] demonstrated that the acceptability of the vaginal rings varied according to the intended indication, accessibility of other dosage forms, and expected risk. Generally, the acceptance of vaginal ring is highest when indicated for the treatment of GSM compared to contraception. Also, many studies compared the acceptability of estradiol vaginal rings to other estrogen dosages or a placebo. One study found that there is no statistically significant difference in the acceptability between Estring® and Vagifem®. The finding showed that 85% of Vagifem® and 83% of Estring® treated patients reported the products as excellent or good, while only 4% of Vagifem® treated patients and 3% Estring® treated patients reported the products as bad or unacceptable.[90] Other studies[88,89,91,92] showed more significant acceptability for Estring® compared to Premarin® cream, estriol cream, and estriol vaginal pessaries. This high acceptance over creams and pessaries may be attributable to the leakage and messiness associated with these dosage forms. The acceptability of Femring® was also assessed by Speroff and coworkers in a multi-center, double blind, randomized, placebo-controlled phase 3 trial. The vaginal ring showed significant improvement in urogenital and vasomotor symptoms, mild-moderate adverse events, and was well tolerated. The majority of women found the ring was easy to remove and reinsert and only a small number of participants reported discomfort during intercourse.[95] Although the estradiol vaginal rings are associated with a high acceptance rate, more research is needed to clarify the impact of the ring's physical features such as flexibility, outer diameter, and cross-sectional diameter on patient acceptability.

Vaginal Creams

Vaginal creams as a delivery system are associated with several limitations and drawbacks, such as being messy, dosage leaks, and sometimes not offering the precise dose.[145] Estrace® is a 0.01% estradiol vaginal cream and was approved by the FDA in 1984 for the treatment of vulvovaginal atrophy in postmenopausal women.[6] The recommended dose schedule for Estrace® cream is 2-4 grams applied daily for the first two weeks with a gradual decrease of the dose to half over 1-2 weeks. Afterwards, one gram is applied once to three times per week as a maintenance dose.[106] Little data and information exist on the acceptability and systemic absorption of estradiol after using Estrace® cream. However, several phase 3 clinical studies (NCT03294538,[100] NCT03332303,[146] NCT02995694,[147] and NCT02195986[148]) were performed to evaluate and compare the therapeutic equivalence and safety of generic 0.01% estradiol vaginal creams to the FDA listed Estrace® vaginal cream.

A study by Rigg et al.[149] evaluated and compared the systemic estradiol absorption of two cream concentrations (2 mg or 0.2 mg estradiol per 2g cream) with Premarin® cream. The products were evaluated in six women with the sequence determined by a latin square design and the serum estradiol and estrone levels measured using a radioimmunoassay. In the 2 mg dose, the serum estradiol concentrations were significantly raised ($p < 0.05$) within 15 min and the C_{max} (527pg/ml) was reached at 4 hours after application. The levels thereafter decreased gradually but remained 19 times greater than the baseline level at 24 hours. The 0.2 mg dose increased serum estradiol levels ($p < 0.01$) within 30 min and the C_{max} (80pg/ml) was reached at 4 hours, with the concentration decreasing to 34pg/ml at 24 hours. Contrary, Premarin® resulted in a slow rising in systemic estradiol and the C_{max} (33pg/ml) was observed 6 hours after application, with the concentration decreasing at 24 hours to levels similar to the baseline level. Another study measured and compared the systemic absorption of estradiol and estrone following the application of 0.01% Estrace® cream and 1.25 mg Premarin® cream in 29 postmenopausal women for two weeks. The Estrace® cream showed increased serum estradiol estrone levels to about 4-fold and 2.5-fold, respectively, within 12 hours of first application. The levels of both estradiol and estrone after two weeks of daily application were found to be the same levels as 12 hours after initial application. Additionally, the levels of estradiol and estrone in the Premarin® treated group at 12 hours and after two weeks were found to be significantly lower than that of the Estrace® cream. These findings indicated that the topical vaginal estradiol cream is rapidly absorbed into the systemic circulation and can result in appreciable blood levels of estrogen.[99]

Safety of Vaginal Creams

With the vaginal tablet and ring dosing regimens, patients receive approximately 1.14 mg and 2.74 mg of estradiol over a one-year treatment period, respectively, while vaginal cream exposes the patient to higher doses of estrogen[66], with the use of 0.01% estradiol cream (considered as high-strength) associated with high systemic exposure. Consequently, the European Medicines Agency in 2020 recommended that the use of the 0.01% estradiol cream be limited to a 4-week period.[7,150] In addition, US FDA guidance recommends the use of appropriate low doses of estrogens in order to reduce systemic estrogen exposure.[6,101] Two randomized, double-blind, placebo-controlled studies from the same working group[6,101] examined the efficacy and safety of investigational very low dose 0.003% estradiol creams (three times lower than Estrace®). Both trials found that the 0.003% cream is effective and well tolerated. The authors further identified that the daily application of estradiol cream for two weeks followed by three times per weeks, resulted in a significant improvement on superficial cells, decreased parabasal cells, reduced vaginal pH, decreased vaginal dryness severity, and improvement in dyspareunia. In terms of safety, there is no significant difference between estradiol and the placebo groups except for vulvovaginal mycotic infections, which were more frequent with the estradiol group. The 0.003% estradiol cream also provides very low doses (15 μg per application), which ensures effectiveness and minimize systemic absorption. The cream is also compatible with FDA recommendations and similar to that provided by the 10-25 μg approved tablets.[6,101] It was noted that in this study, the majority of participants were of a single demographic, therefore the results of both studies may not be generalized to all ethnic groups. Additionally, the systemic absorption of estradiol was also not measured in any of the studies, although the creams tested contained small amounts of estradiol and systemic exposure would be expected to be negligible. Furthermore, both studies did not report the incidence of messiness and leakage, which may highly impact the acceptance rate, and the safety of the creams, which should be evaluated in long-term studies.

Another study, more recently in 2020[59] evaluated the efficacy of a 25 μg estradiol gel on postmenopausal patients with vaginal atrophy.

Although the findings demonstrated significant improvement in laboratory markers between participants of the estradiol group compared to the placebo group, the most bothersome symptoms of GSM were improved equally with no significant difference between both groups. This may be due to the lubricant effect of K-Y® Jelly, which was used as placebo in the study. Also, in this study, the analysis of serum estradiol level showed no higher concentration of estradiol than the reference level in postmenopausal women. In addition, there was no increase in endometrial thickness and no adverse events reported at Week 4 or Week 8. Another 12-weeks study evaluated the efficacy of various doses of estradiol cream (10, 5, 2.5, and 1.25 μg) for the alleviation of GSM symptoms. The 10 μg dose demonstrated reduced pH and improved vaginal cytology. The systemic estradiol also remained within the postmenopausal range and there was no effect on the endometrium.[151]

The lower urinary tract is rich with estrogens receptors; hence, the application of estrogen is expected to have a useful outcome on urinary tissue, function and symptoms. Several studies have therefore examined the efficacy of estrogen in treatment of overactive bladder and urinary incontinence. The intravaginal estrogen treatment was shown to be more beneficial for the lower urinary tract than oral formulations.[152] Ellington et al. compared the efficacy of Estrace® cream with an oral tolterodine formulation for the treatment of overactive bladder symptoms in a 12-week single-site randomized, open-label trial. The authors found that there is significant improvement in overactive bladder symptoms in both groups from baseline to 12-weeks, with no difference between the two groups of treatment.[152]

Acceptability

The effectiveness of local estrogen therapy is greatly affected by the adherence to the treatment with deviation from the recommended dosing schedule increasing the probability of treatment failure. The treatment adherence may also be attributable to a fixed dosing schedule, the formulation being less messy, and the convenience of the system.[9] Locally-administered vaginal estrogens creams however are associated with many limitations including being messy, uneven application intervals, with many patients finding the cream difficult to apply, especially in the elderly.[92,94] In a retrospective study[9], comparing adherence of postmenopausal women with vulvovaginal atrophy to vaginal estrogen therapy, it was found that women who used Premarin® or Estrace® cream terminated treatment earlier than those on the 10 μg Vagifem® tablet. The investigators followed 30,197 women, where 40.4% started the treatment with Premarin® cream, 38.3% initiated with Estrace® cream, and 21.3% with Vagifem® tablets. Throughout a one-year follow-up, 57.8% of Vagifem® users terminated the therapy after the first prescription fills in contrast to 86.2% - 89.4% of the cream users ($p < 0.0001$). The average treatment period was 44.6 to 48.1 days for the creams compared to 103.4 days for Vagifem® ($p < 0.0001$).

Advanced Estradiol Drug Delivery Systems for Menopausal Symptoms

With the large number of developed and marketed oral tablets, vaginal rings and creams, advanced platforms have been researched to enhance patient treatment, safety, and acceptability. Hormonal

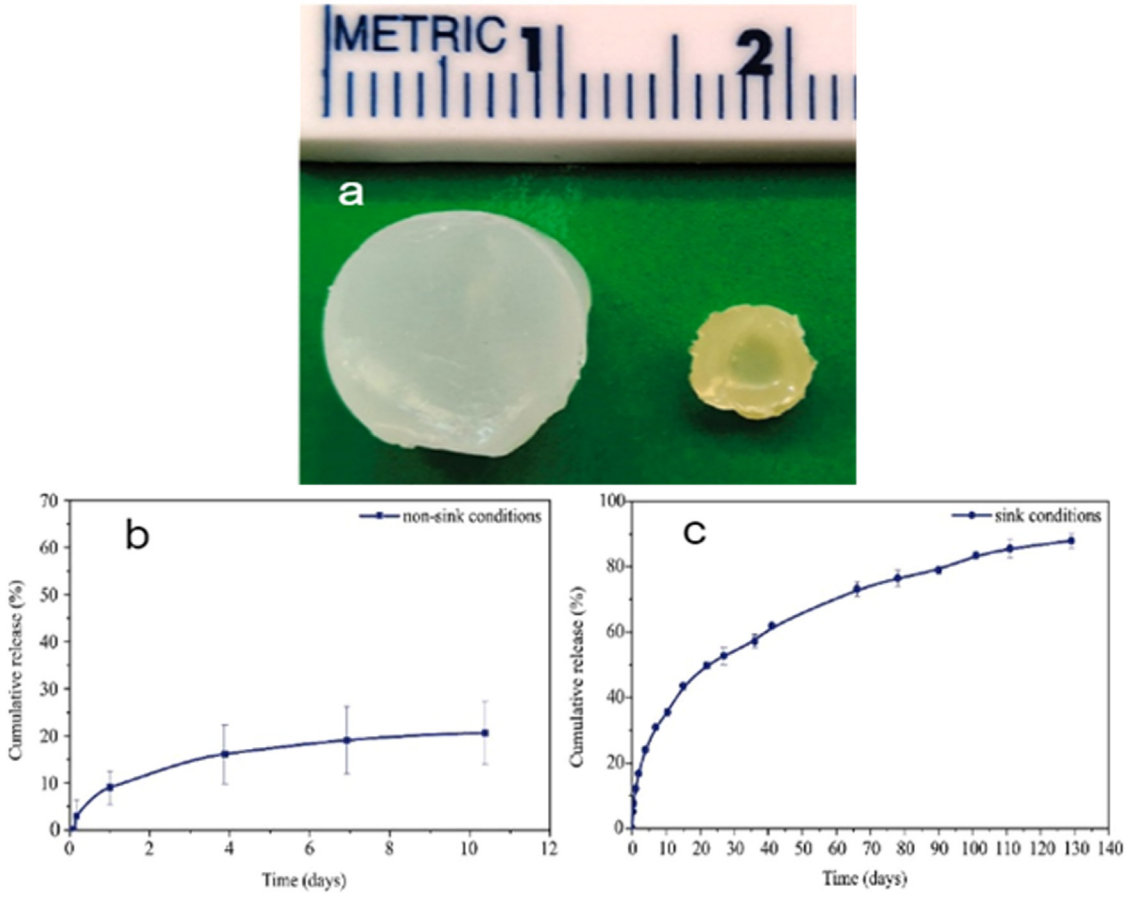

Figure 7. (a) Morphology of silk fibroin hydrogel (left) and xerogel (right) samples with estradiol; *in vitro* release profiles of estradiol from xerogel delivery systems in (b) non-sink and (c) sink conditions (arithmetic means and standard deviations of n = 3). Adapted with permission from Križman et al.[45]

replacement therapy has also been investigated to be delivered through various routes including orally, nasally, dermally, vaginally, and intramuscularly, where the dose regimen could be customized to each patient.[153] The availability of versatile polymers and their incorporation into drug delivery systems has also made a remarkable advance in this field of research. Several of these polymers have been employed in the formulation of vaginal delivery systems to address practical challenges such as leakage and imparting sustained controlled release.[17] Other systems have additionally been developed for the delivery of estradiol topically. Estradiol transdermal spray solutions are considered appropriate delivery systems for the alleviation of menopausal symptoms, especially for women with diabetes mellitus hypertriglyceridemia and those with altered liver function. These delivery systems offer better bioavailability, steady estrogen levels and estradiol/estrone ratio over extended periods of time and are associated with low adverse events compared to oral estrogen.[153] Additionally, the transdermal spray delivery systems are free of shortfalls related to traditional transdermal estradiol delivery systems.[154] One novel transdermal spray solution is Lenzetto® (Gedeon Richter Plt., Hungary) which contain 1.53 mg of estradiol per 90 μl one spray dose.[153,154] The Lenzetto spray container contains 95% ethanol, octisalate (as skin penetrating agent), in addition to the 17β-estradiol. The application of the dose is controlled to the specific area by using a plastic housing, which is controlling the angle and distance of the sprier from surface of the skin.[154]

Additionally, in a study by Križman et al.,[45] the practicability of silk fibroin xerogels as an implantable drug delivery system for continuous controlled-released estradiol delivery in hormone replacement therapy was investigated. The system exhibited sustained *in vitro* release for more than four months (Fig. 7) and had a biodegradation pattern suitable for the extended term delivery of estradiol. The system could also offer an attractive alternative to conventional systems and could be useful in future clinical applications. These systems, while not administered vaginally, have shown the increased potential of developing advanced drug delivery platforms for the treatment of GSM.

Future Perspectives

Although there is significant research data and reports studying and evaluating vaginal estradiol delivery systems, there is limited research data evaluating and comparing the Estrace® vaginal cream with other vaginal estrogens preparations. Future studies are also needed to elucidate the pharmacokinetics, efficacy, safety and patient's acceptability of the Estrace® vaginal cream. Additionally, there is a need for studies of more than one year to evaluate the safety of vaginal estradiol delivery systems for long term use.

Currently available vaginal estradiol delivery systems have been shown to be equally effective in alleviating GSM symptoms, however, there are still some challenges and limitations which must be overcome. Creams need an applicator for use, are messy, leaky, and associated with excessive discharge, while tablets may remain intact or not completely dissolved. Creams, tablets, and capsules additionally require repeatable insertions or applications. Rings are designed to have a controllable release of estradiol over three months but may dislodge and their insertion and removal may be difficult and requires a trained health care practitioner. These challenges could lead to decreased levels of patient comfort and, as a consequence, the treatment adherence reduces over time. Hence, a delivery system based on suitable materials, free of limitations, and administered less frequently, can ensure increased patient acceptability and greater control and treatment of GSM.

Conclusions

GSM is a common, chronic, under-diagnosed, and under-treated condition due to decreased estrogen levels throughout menopause. The treatment of GSM should be continued for extended periods of time as long as the condition persists. Local vaginal low dose estrogen therapies are employed for treatment of mild to severe GSM when over-the-counter therapies fail to control symptoms. A variety of pharmaceutical delivery systems of low dose estradiol in the form of tablets, softgel capsules, creams, and rings are approved and available and are in use for the site-specific release of estradiol to vaginal tissues. These products, with the exception of the vaginal rings which are applied every three months, are administered locally in a complicated dose regimen, where multiple administrations are often required per week. All forms are effective and provide rapid improvement in the subjective and objective symptoms of GSM, but not the vasomotor symptoms, except when using the high dose Femring®. Additionally, most local estradiol containing formulations exhibit negligible to low systemic estradiol exposure in levels that do not exceed the normal postmenopausal range. This low absorption of estradiol, associated with non-significant differences compared to a placebo in the incidence of endometrium stimulation, is free of the risk of serious side effects of systemic estrogen treatment. The choice of a particular local estradiol delivery system, however, is highly determined by the patient's acceptability and preference, and this should be considered in the development of future pharmaceutical systems for the treatment of GSM.

Conflicts of Interest

The authors do not declare any conflicts of interest.

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