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Vaginal tamoxifen for treatment of vulvar and vaginal atrophy: Pharmacokinetics and local tolerance in a rabbit model over 28 days

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ABSTRACT

Vaginally delivered tamoxifen is being developed as alternative to estrogen-based therapies for the treatment of vulvar and vaginal atrophy (VVA) symptoms in subjects at high risk for breast cancer, undergoing treatment for breast cancer with aromatase inhibitors or are breast cancer survivors. Tamoxifen (1 or 20 mg) was administered intra-vaginally to female rabbits once-daily over a 28-day period to assess its pharmacokinetics, systemic exposure and local vaginal tolerance. Plasma samples were taken to assess concentrations of tamoxifen and its metabolites 4-hydroxytamoxifen and *N*-desmethyltamoxifen over the first day of vaginal administration and following the last dose on Day 28. In-life observations included evaluation of the vaginal region for signs of irritation. At necropsy, vaginal irritation was assessed by the method of Eckstein which reflect collective histopathological grading of four parameters within the vagina including epithelial morphology, leukocytic infiltration, congestion, and edema. Uterine effects of vaginal tamoxifen were also assessed. Plasma concentrations of tamoxifen were higher following administration of 20 mg tamoxifen compared to 1 mg tamoxifen at both Day 1 and Day 28. The metabolite 4-hydroxytamoxifen could not be detected on Day 1 and concentrations were low at Day 28 at the 1 mg tamoxifen dose. 4-Hydroxytamoxifen concentrations were low, but detectable at Day 1 and 28 following administration of 20 mg tamoxifen. The metabolite *N*-desmethyltamoxifen was undetectable at the 1 mg and 20 mg doses on Day 1; it remained undetectable at the 1 mg tamoxifen dose at Day 28. *N*-desmethyltamoxifen was detected over the first 8 h of Day 28 then fell below the quantitation limits. There was little to no vaginal or systemic accumulation of tamoxifen following once-daily dosing for 28 days. Tamoxifen accounted for more than 85% of the total systemic exposure compared to its metabolites, 4-hydroxytamoxifen, and *N*-desmethyltamoxifen. There was essentially no detectable vaginal irritation evident over the course of the study. At necropsy the individual Eckstein scores (maximum score of 16) of the proximal, mid, and distal vagina of females in the 1 mg and 20 mg dose groups were generally comparable in both groups and ranged from minimal to mild magnitude (1 mg dose group: ranging from 1 to 3 in the proximal vagina, 4 to 5 in the mid vagina, and 3 to 7 in the distal vagina; 20 mg dose group: ranging from 3 to 5 in the proximal vagina, 4 to 7 in the mid vagina, and 4 to 5 in the distal vagina). Overall, tamoxifen was absorbed and metabolized following vaginal administration and vaginal irritation was minimal to none at both doses.

1. Introduction

Tamoxifen, a selective estrogen receptor modulator (SERM), has been prescribed widely as an adjuvant treatment for breast cancer since the mid-1970s (Jordan, 1990). Tamoxifen has been approved for therapy in postmenopausal node positive women as well as hormone receptor positive postmenopausal and node negative breast cancer patients (EBCTCG, 2005), breast cancer prevention (Fisher et al., 1998), and ductal carcinoma in situ (Fisher et al., 1999). SERMs like tamoxifen possesses both estrogen receptor antagonist properties as well as agonist properties depending on the tissues being studied (Patel and Bihani, 2018; Wardell et al., 2014). Metabolites of tamoxifen including 4-hydroxytamoxifen and *N*-desmethyltamoxifen are also estrogen receptor antagonists (Etienne et al., 1989; Löser et al., 1985).

Tamoxifen has been found to exert estrogenic activity on the vagina and endometrium (Ferrazzi et al., 1977; Varras et al., 2003). A number

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of studies have been published describing the effects of orally administered tamoxifen on vaginal epithelium in postmenopausal women being treated for breast cancer (Bertolissi et al., 1998; Boccardo et al., 1981; Ellmén et al., 2003; Lahti et al., 1994; Love et al., 2000; Miodrag et al., 1991; Shiota et al., 2002; Vardy et al., 2003). These effects include improvement in vaginal maturation index or in vaginal pH. Historically, estrogen is administered either topically or orally to treat symptoms of vulvar and vaginal atrophy (VVA). However, estrogen therapy is contraindicated in patients with a known, suspected or history of cancer of the breast. There is limited data regarding the safety of estrogen replacement in women who are BRCA2-positive. Therefore, tamoxifen potentially represents a nonhormonal VVA treatment option.

Vaginal administration of tamoxifen could help avoid the well-known systemic effects of orally administered tamoxifen (Neven and Vernaeve, 2000; Varras et al., 2003) while maintaining the vaginal estrogenic effects that should relieve symptoms of VVA. This concept has been explored recently in a pilot study wherein weekly administration of tamoxifen over three months as a vaginal suppository in postmenopausal women with VVA showed improvements in vaginal pH and vaginal dryness symptoms (Chollet et al., 2019).

To create a more acceptable vaginal product, development of a vaginal insert (i.e., a tablet) was explored. At the same time, it is important to examine the absorption and metabolism characteristics of vaginally applied tamoxifen as well as the local effects on the vagina and reproductive tissues. To that end, a study in rabbits was conducted to assess the pharmacokinetics and local tolerance of vaginally applied tamoxifen. Two doses of tamoxifen were evaluated: 1 mg or 20 mg (the higher dose is approximately equal to the standard oral dose used to treat women with breast cancer). Both doses were administered along with the tablet excipients in the form of a granulation. The results of this study in rabbits is the subject of this report.

2. Materials and methods

2.1. Formulation

Tamoxifen citrate, USP, was obtained from Plantex Ltd., Chemical Industries, Netanya, Israel. The drug substance had a d_{10} of 0.7 μm and a d_{90} of 29.7 μm. All other excipients were either USP or NF grade. The formulations consisted of tamoxifen citrate, lactose, Starch 1500, microcrystalline cellulose, Crospovidone XL, hydroxypropyl methylcellulose (Methocel E5P), and magnesium stearate (see Table 1 for the composition of each blend). The first five ingredients were blended (GMX-Lab Micro, Freund-Vector, Marrion, IA) followed by wet granulation using the Methocel E5P in water as the granulation medium. The granulation was performed in the GMX-Lab Micro unit followed by drying at 40 °C. The granulation was sieved through 30 mesh (595 μm) followed by milling in a Comill Model 197 (Waterloo, ON, Canada). The milled material was also sieved through the 30-mesh screen. Finally, the magnesium stearate was blended into the granulation. In addition, the same granulation was prepared but did not contain tamoxifen citrate. This granulation was used as the placebo control.

Table 1
Composition of the 1 and 20 mg tamoxifen test articles.

Component	Function	(mg)	
		1 mg Tamoxifen	20 mg Tamoxifen
Tamoxifen citrate	Active	1.5	30.4
Lactose	Diluent	38.0	25.4
Starch 1500	Diluent	16.3	10.9
Microcrystalline cellulose	Diluent	18.2	7.3
Crospovidone XL	Disintegrant	3.2	3.2
Methocel E5P (hydroxypropyl methyl cellulose)	Binder	2.2	2.2
Magnesium stearate	Lubricant	0.6	0.6

2.2. Preparation of test articles

The granulations prepared as described above were used to prepare test articles to be administered to the rabbits with a final concentration of 1.5 mg tamoxifen citrate (equivalent to 1.0 mg tamoxifen in 80 mg blend) or 30.4 mg tamoxifen citrate (equivalent to 20 mg tamoxifen in 80 mg of blend) in 1.0 mL vehicle (0.9% sodium chloride). Fresh test articles were prepared once weekly. The placebo control was prepared using the placebo granulation (80 mg) in 1.0 mL of 0.9% saline.

2.3. Rabbit study

The animal study was conducted by an American Association for Accreditation of Laboratory Animal Care (AAALAC) accredited contract research organization facility (MPI Research, now Charles River Company, Mattawan, MI). The study was conducted in compliance with the US Food and Drug Administration (FDA) Good Laboratory Practices (GLP) Regulations and the US Department of Agriculture (USDA) Animal Welfare Act. The study protocol was approved by the MPI Research IACUC. A total of 26 female experimentally naïve New Zealand White Hra:(NZW)SPF albino rabbits (approximately 7.5 months of age at receipt) were received from Covance Research Products, Greenfield, IN. During the 9-day acclimation period, the animals were observed daily with respect to general health and any signs of disease.

Animals were acclimated to Elizabethan collars over three consecutive days. Exposure to the collars began on the first day for approximately 2 h with the time in the collars increased each day by approximately 2 h until approximately 6 h was reached. Using a standard, by weight, measured value randomization procedure, 24 female animals (weighing 2.84 to 3.74 kg at randomization) were assigned to the control and treatment groups identified in Table 2.

Animals were assigned an animal number to be used in the Provantis™ data collection system. Each animal was implanted with a microchip bearing a unique identification number. The combination of individual animal number, implant number, and study number create a unique identification for each animal. Cages were identified by animal number, study number, group number, and sex.

The animals were individually housed in suspended, stainless steel, slatted floor cages. Animal enrichment was provided according to SOP. Fluorescent lighting was provided for approximately 12 h per day. The dark cycle was interrupted intermittently due to study-related activities. Temperature and humidity were continuously monitored, recorded, and maintained to the maximum extent possible within the protocol-designated ranges of 16 to 22 °C and 30 to 70%, respectively. Lab Diet® (Certified Rabbit Diet #5322, PMI Nutrition International, Inc.) was limited upon arrival and was increased daily until feeding was approximately 125 g/animal/day. The lot number from each diet lot used for this study was recorded.

The vehicle, 0.9% Sodium Chloride for Injection, USP, placebo, and

Table 2
Group assignments.

Group number	Treatment	Tamoxifen (mg)	Number of female animals[b]
1	Vehicle Control[a]	0	6
2	Placebo Control	0	6
3	Low Dose Tamoxifen	1	6
4	High Dose Tamoxifen	20	6

[a] 0.9% Sodium chloride for injection, USP.
[b] Two animals/sex/group were maintained for a 14-day recovery period.

test articles, 1 mg tamoxifen, or 20 mg tamoxifen, were administered once daily for up to 28 consecutive days during the study via intravaginal administration at a dose volume of 1 mL/animal.

A 1 mL syringe (BD Luer-Lok Tip REF 309628) and catheter (sterile disposable roundtip polyurethane umbilical style catheter with luer, 0.058″ ID × 0.096″ OD × 88 mm (Instech Solomon, catalog number FTP-PU-88MM CUS) were used to administer the formulations. The placebo and test article were withdrawn from stirred formulations. Saline was used to lubricate the tip of the catheter before dosing, as needed. Each animal was removed from the cage and held in a position such that the vaginal opening was easily accessible. The catheter (88 mm in length) was gently inserted into the vaginal opening of the animal to a depth of approximately 7–8 cm so that the vehicle, placebo, or test article was not washed out by urine. Immediately after dosing, the vagina/vulva was held closed for 1 min. Elizabethan collars were placed on the animals following dosing for 6 h (± 30 min).

All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily. On occasion, veterinary consultations were conducted during the course of the study. All treatments and observations were recorded. There was no observed leakage indicating the all the dose was available for absorption from the rabbit vagina.

A detailed clinical examination of each animal was performed as described previously (Weiss et al., 2019).

The vaginal region of the animals was evaluated for signs of irritation using a modified Draize scoring system (Draize et al., 1944). Evaluations were conducted prior to each daily dose and daily during the recovery period. Any abnormalities observed during this examination were documented as a detailed clinical observation. A score of "1" or above was confirmed by a member of the veterinary staff.

Clinical pathology evaluations were conducted pretest (all animals), prior to terminal necropsy (three or four animals/group, survival permitting), and prior to the recovery necropsy (two animals/group). The animals had access to drinking water and food prior to sample collection.

2.4. Pharmacokinetics

Blood samples (approximately 1 mL) were collected from all surviving animals via the jugular vein for determination of the plasma concentrations of the test article. Samples were collected at pre-dose and at 0.5, 1, 3, 8, and 24 h post-dose relative to dosing on Days 1 and 28. The animals were not fasted prior to blood collection. Samples were placed in tubes containing K_2EDTA as an anticoagulant on wet ice until centrifuged. The samples were contained in tightly capped, pre-labeled, plastic vials and were stored frozen at −60 to −90 °C until analyzed. The vial label included the study number, relative study day, animal number, and the date and time interval of collection.

Plasma samples were analyzed using a liquid chromatography-mass spectrometry/mass spectrometry method validated according to bioanalytical method guidelines by MPI Research (now Charles River Co., Mattawan, MI). The method was designed to measure tamoxifen, 4-hydroxytamoxifen, and *N*-desmethyltamoxifen. These two metabolites are commonly found following oral administration of tamoxifen to humans and rats (Lien et al., 1989; Lien et al., 1991) The standard curve range for tamoxifen was 0.100 (LLOQ) to 100 ng/mL (ULOQ); the range for 4-hydroxytamoxifen was 0.100 (LLOQ) to 100 ng/mL (ULOQ); and the range for *N*-desmethyltamoxifen was 0.500 (LLOQ) to 500 ng/mL (ULOQ). Concentrations below the LLOQ were set to zero for pharmacokinetic analyses.

Pharmacokinetic parameters were determined by non-compartmental methods using Phoenix™ WinNonlin® (Version 6.3.0) Model: Plasma Data, Extravascular Administration for individual serial sample collection data (Pharsight Corporation). Area under the curve values were calculated using the linear trapezoidal rule with linear interpolation. Nominal sample collection time was used for the analysis unless the actual sample collection time exceeded the allowable collection window for the respective time; in which case, the actual sample collection time was used. Pharmacokinetic parameters determined were defined as follows. C_{max}: Maximum observed concentration occurring at T_{max}; T_{max}: Time of maximum observed concentration; $AUC_{0\text{-}t}$: Area under the curve from the time of dosing to the time of the last observation.

2.5. Necropsy

Necropsy examinations were performed on all surviving animals at the scheduled terminal and recovery necropsies. The animals were euthanized by an intravenous overdose of sodium pentobarbital solution followed by exsanguination via transection of the femoral vessels or abdominal vena cava. The animals were examined carefully for external abnormalities including palpable masses. The skin was reflected from a ventral midline incision and any subcutaneous masses were identified and correlated with antemortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities. The organs were removed, examined, and, where required, placed in fixative.

Body weights and protocol-designated organ weights were recorded for all surviving animals at the scheduled necropsies and appropriate organ weight ratios were calculated (relative to body and brain weights). Paired organs were weighed together.

Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed on protocol-designated sections of tissues. The vagina (proximal, mid, and distal) and uterus with cervix were determined to be potential target organs and were examined for all animals. The slides were examined by a board-certified veterinary pathologist. A four-step grading system was utilized to define gradable lesions for comparison between dose groups.

A semi-quantitative grading system (Eckstein et al., 1969) was used for scoring microscopic changes in sections of the vagina (proximal, mid, and distal) from scheduled necropsy animals. A collective score for the four parameters of 1 to 4 was considered minimal, 5 to 8 mild, 9 to 11 moderate, and 12 to 16 severe.

2.6. Statistical analyses

Statistical analysis of data include descriptive statistics, including means, standard deviations (SD), group size for each group and time period (continuous endpoints), and either medians or incident counts for each group and time period (categorical endpoints). In addition, determination of statistical comparisons (*t*-test) of pharmacokinetic parameters were conducted using GraphPad Prism Version 8.2.

3. Results

3.1. Pharmacokinetics

The mean plasma concentrations of tamoxifen, 4-hydroxytamoxifen, and *N*-desmethyltamoxifen (see Fig. 1 for chemical structures) are shown in Figs. 2, 3, and 4, respectively. Each figure shows the concentration over time of each analyte following a single vaginal dose of 1.0 or 20 mg tamoxifen (Day 1) and after 28 days of once-daily vaginal administration of 1.0 or 20 mg tamoxifen (Day 28). The concentrations of tamoxifen were substantially higher than either of the two metabolites measured. The concentrations of tamoxifen on Day 1 were on average higher than after 28 days of once daily dosing. The concentrations of both metabolites were relatively low and in many instances were below the LLOQ.

The pharmacokinetic parameters evaluated (T_{max}, C_{max}, and $AUC_{0\text{-}t}$) are shown in Table 3. Also shown in Table 3 are the ratios of C_{max}/Dose and $AUC_{0\text{-}t}$/Dose. Vaginal tamoxifen had a relatively short T_{max} after the first and last dose (0.5 to 1.0 h). T_{max} of the metabolites was

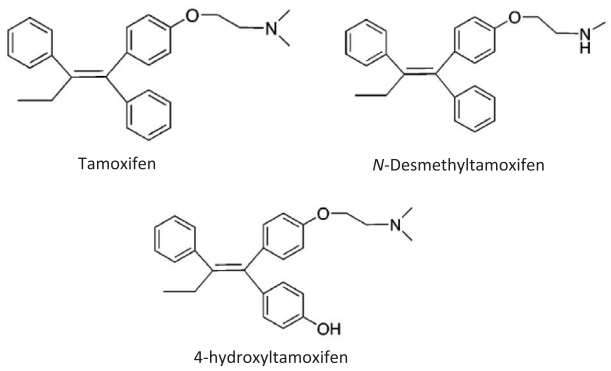

Fig. 1. Chemical structures of tamoxifen, *N*-desmethyltamoxifen, and 4-hydroxytamoxifen.

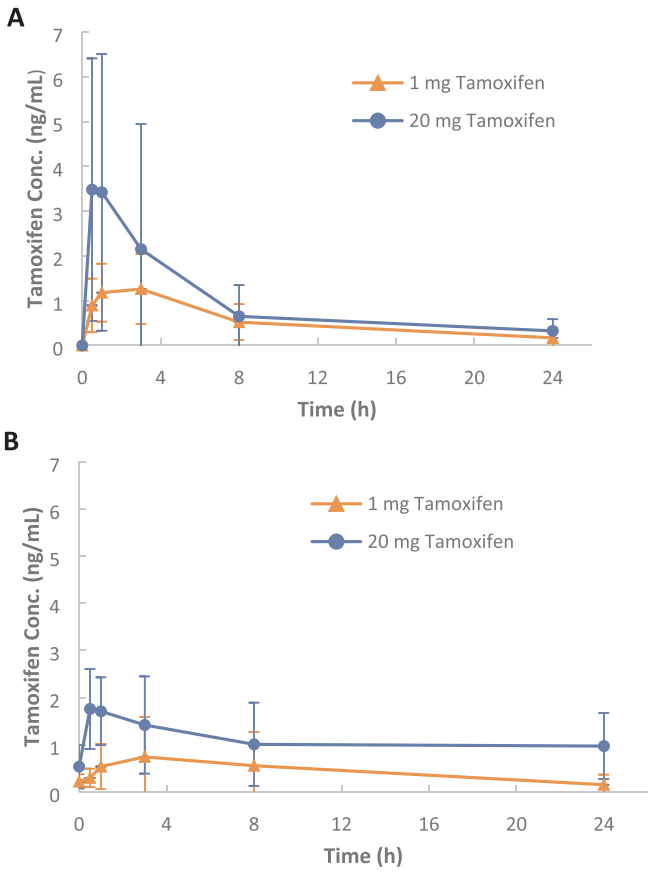

Fig. 2. Tamoxifen plasma concentrations following 1 mg or 20 mg tamoxifen vaginally to rabbits. A: plasma concentrations over Day 1; B: plasma concentrations following 28 days once-daily administration. Values are mean ± SD (n = 6) except the 1 mg tamoxifen group on Day 28 where n = 5.

variable (1, 8, or 13.5 h). C_{max} values were highest for tamoxifen and was higher following the 20 mg dose compared with 1 mg dose at both Day 1 and Day 28. The C_{max} values for the two metabolites were considerably lower and in some cases were zero due the inability to detect them. The $AUC_{0\text{-}t}$ of tamoxifen ranged from 9 to 26.5 ng*h/mL and were higher at Day 28 compared with Day 1. Metabolite $AUC_{0\text{-}t}$ were considerably lower compared with those of tamoxifen (see Table 3). The ratio of C_{max} and $AUC_{0\text{-}t}$ to dose when measurable was always substantially lower at Day 28 compared with Day 1. Based on mean $AUC_{0\text{-}t}$ as a measure of exposure, tamoxifen exhibited less than dose-proportional systemic exposure on Day 1 and Day 28 over the dose range of 1 to 20 mg.

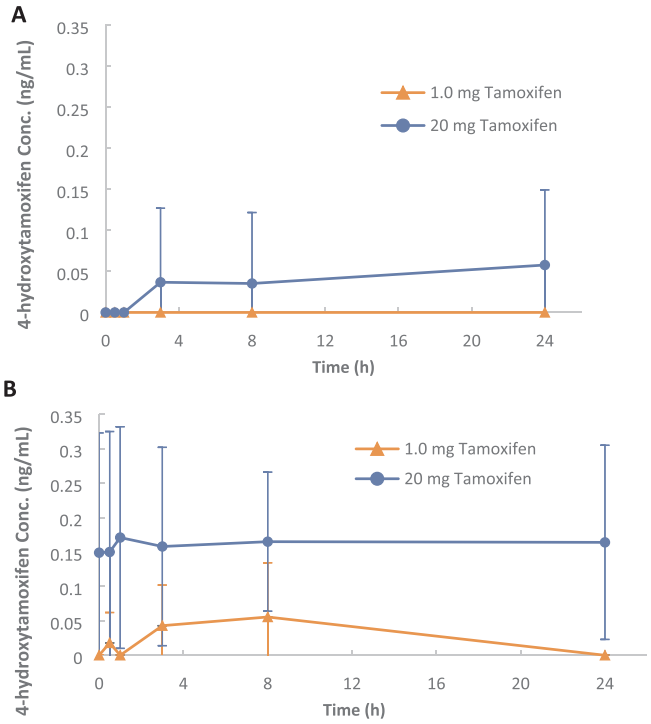

Fig. 3. 4-Hydroxytamoxifen plasma concentrations following 1 mg or 20 mg tamoxifen vaginally to rabbits. A: plasma concentrations over Day 1; B: plasma concentrations following 28 days once-daily administration. Values are mean ± SD (n = 6) except the 1 mg tamoxifen group on Day 28 where n = 5.

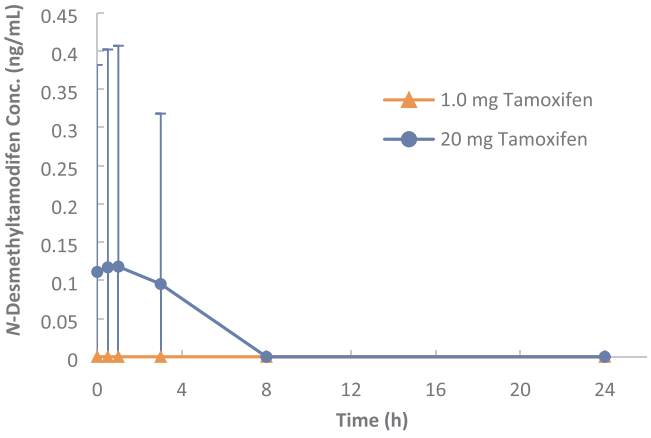

Fig. 4. *N*-Desmethyltamoxifen plasma concentrations following 1 mg or 20 mg tamoxifen vaginally to rabbits following 28 days once-daily administration. Values are mean ± SD (n = 6). There were no detectable concentrations of *N*-desmethyltamoxifen following the either the 1 mg or 20 mg dose of tamoxifen on Day 1.

Exposure to tamoxifen was generally lower on Day 28 than on Day 1 in the 1.0 mg dose group and slightly higher in Day 28 than on Day 1 in the 20 mg dose group. This finding indicates that little to no accumulation of tamoxifen occurred following once daily vaginal administration of 1 or 20 mg tamoxifen. Accumulation of 4 hydroxytamoxifen could not be evaluated in the 1.0 mg dose group although some accumulation of 4-hydroxytamoxifen occurred in the 20 mg dose group since the ratio of 4-hydroxytamoxifen $AUC_{0\text{-}t}$ for Day 28/Day 1 was 4.08. Accumulation of *N*-desmethyltamoxifen could not be evaluated in either dose group.

Exposure from tamoxifen was greater than from 4-hydroxytamoxifen and *N*-desmethyltamoxifen on both Day 1 and Day 28. Ratios of mean 4-hydroxytamoxifen to tamoxifen $AUC_{0\text{-}t}$ values could

Table 3
Mean (standard deviation, SD) pharmacokinetic parameters for tamoxifen, 4-hydroxytamoxifen, and *N*-desmethlytamoxifen on Day 1 and Day 28 following vaginal administration of 1.0 or 20 mg tamoxifen.[a]

Analyte	Tamoxifen Dose (mg)	T_{max} (h)[b]	C_{max} (ng/mL)	AUC_{0-t} (ng*h/mL)	C_{max}/Dose [(ng/mL)/(mg/kg)]	AUC_{0-t}/Dose [(ng*h/mL)/(mg/kg)]
Day 1						
Tamoxifen	1	1	1.44 (0.786)	13.2 (8.24)	1.44 (0.786)	13.2 (8.24)
	20	0.5	3.65 (2.96)[c]	23.0 (23.7)[d]	0.182 (0.148)	1.15 (1.19)
4-Hydroxy-tamoxifen	1	–	0 (0)	0 (0)	0 (0)	0 (0)
	20	13.5	0.0708 (0.110)	0.963 (1.69)	0.00354 (0.00549)	0.0481 (0.0845)
N-Desmethyl-tamoxifen	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	20	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Day 28						
Tamoxifen	1	1.0	0.824 (0.819)	8.85 (11.6)	0.824 (0.819)	8.85 (11.6)
	20	0.5	2.33 (0.667)[e]	26.5 (16.4)[f]	0.116 (0.0333)	1.32 (0.820)
4-Hydroxy-tamoxifen	1	8	0.0463 (0.0739)	0.621 (0.985)	0.0463 (0.0739)	0.621 (0.985)
	20	1	0.219 (0.140)[g]	3.93 (2.89)[h]	0.0109 (0.0071)	0.196 (0.145)
N-Desmethyl-tamoxifen	1	–	0 (0)	0 (0)	0 (0)	0 (0)
	20	1	0.118 (0.289)	0.566 (1.39)	0.00589 (0.0144)	0.0283 (0.094)

[a] $n = 6$ for Day 1 and $n = 5$ for Day 28.
[b] Median values reported.
[c] Difference between doses was insignificant ($p = 0.11$).
[d] Difference between doses was insignificant ($p = 0.36$).
[e] Difference between doses was significant ($p = 0.013$).
[f] Difference between doses was insignificant ($p = 0.085$).
[g] Difference between doses was significant ($p = 0.038$).
[h] Difference between doses was significant ($p = 0.042$).

not be determined in the 1.0 mg tamoxifen dose group and was 0.0419 in the 20 mg dose group on Day 1. Ratios of mean 4-hydroxytamoxifen to tamoxifen AUC_{0-t} values ranged from 0.0702 to 0.148 on Day 28. Ratios of mean *N*-desmethyltamoxifen to tamoxifen AUC_{0-t} values could only be determined in the 20 mg tamoxifen dose group on Day 28 (0.0214). These findings indicated that tamoxifen accounted for approximately 85% or more of the total systemic exposure to tamoxifen, 4-hydroxytamoxifen, and *N*-desmethyltamoxifen following once daily intravaginal administration of 1 to 20 mg tamoxifen for 28 days to female rabbits.

3.2. In-life observations

There were no test article-related mortalities evident on study. All animals survived to their scheduled terminal necropsy on Day 29 or recovery necropsy on Day 42. One animal administered the 1.0 mg tamoxifen was euthanized *in extremis* on Day 28 due to complications from the clinical pathology blood collection.

There was no test article-related vaginal irritation evident on study as all scores were 0 through Day 28 according to Draize method (Draize et al., 1944). On Day 29 a few animals had a score of 1 across all dose groups. Overall, low and high doses of tamoxifen and the placebo control did not appear to be any more irritating than the vehicle control. Sporadic findings of mild erythema and mild to moderate edema were observed in 1 or 2 of 6 animals in the vehicle control, placebo control, and low dose tamoxifen groups during the 4-week dose period. This was likely the result of the dosing procedure. There were no signs of vaginal irritation during recovery period.

Test article-related body weight effects were observed during the 4-week dose period groups administered low and high doses of tamoxifen. Animals administered the low dose of tamoxifen had a mean body weight gain of 2% and animals administered the high dose of tamoxifen had a loss of 2% over the 4 weeks compared to the vehicle control or placebo control groups which gained a mean of 7% or 5%, respectively. During the recovery period both tamoxifen treated groups gained weight with a greater gain observed in the high dose tamoxifen group.

3.3. Postmortem examinations

Vaginal irritation scores as assessed by the method of Eckstein (Eckstein et al., 1969) reflected the collective histopathological grading of four parameters within the vagina including epithelial morphology, leukocytic infiltration, congestion, and edema.

At the terminal necropsy, the proximal, mid, and distal vagina of females in the 1.0 and 20 mg tamoxifen groups had a slight increase in the individual animal and group mean Eckstein Scores compared to Vehicle Control females. The Eckstein scores of the mid and distal vagina of females in the tamoxifen-treated groups were also slightly increased compared to Placebo Control females. These data are summarized in Fig. 5.

The Eckstein scores of the proximal, mid, and distal vagina of Vehicle Control females at the terminal necropsy were all considered to be of minimal magnitude (individual scores ranging from 1 to 2 in the proximal vagina, 1 to 3 in the mid vagina, and 2 to 3 in the distal vagina). The Eckstein scores of these three regions of the vagina in placebo control females were typically of minimal magnitude, with the exception of scores of the proximal and mid vagina of one female in this group which were of mild severity due to increased leukocytic infiltration (individual scores ranging from 2 to 5 in the proximal vagina, 2 to 7 in the mid vagina, and 2 to 3 in the distal vagina).

The individual Eckstein scores of the proximal, mid, and distal

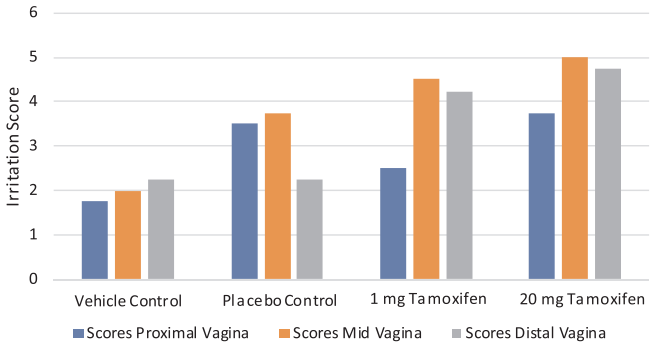

Fig. 5. Summary of histopathologic grading for the rabbit proximal, mid, and distal vagina following 28 days of once daily dosing of the vehicle control, placebo control, 1 mg tamoxifen, or 20 mg tamoxifen ($n = 4$). Maximum possible score is 16 where the collective score for the four parameters of 1 to 4 is considered minimal, 5 to 8 mild, 9 to 11 moderate, and 12 to 16 severe.

vagina of females in the 1 mg and 20 mg dose groups at the terminal necropsy were generally comparable in both groups and ranged from minimal to mild magnitude (1 mg dose group: ranging from 1 to 3 in the proximal vagina, 4 to 5 in the mid vagina, and 3 to 7 in the distal vagina; 20 mg dose group: ranging from 3 to 5 in the proximal vagina, 4 to 7 in the mid vagina, and 4 to 5 in the distal vagina). The higher Eckstein scores of females in the 1.0 and 20 mg dose group compared to the Vehicle Control and Placebo Control groups were primarily associated with alterations in the mucosal epithelium (metaplasia/increased mucification of the proximal and mid vagina, and decreased thickness/flattening/degeneration of the mucosa of the distal vagina), and slight increases in leukocytic infiltration and/or vascular congestion in the mid and distal vagina compared to that observed in control animals. The slight increase in severity of these findings in tamoxifen-treated females was considered test article related but non-adverse due to the overall low magnitude and nature of the changes.

The uterus of two females in the 20 mg tamoxifen group at the terminal necropsy had a mild decrease in the thickness of the endometrial stroma and the uterus of one affected female also had a minimal decrease in the thickness of the myometrium compared to females in the vehicle control and placebo control groups. These findings may have been test article related, however, it is also possible that these findings may have occurred due to variation in tissue sectioning. Therefore, the relationship of these findings to the test article was uncertain. Mild edema noted in the cervix of one female in the placebo control group at the terminal necropsy may have also reflected normal variation, and was not ascribed to test article administration given the absence of similar findings in other treated animals. Microscopic findings in other tissues (oviducts and urethra) noted in control and tamoxifen-treated females at the terminal and recovery necropsies were considered unrelated to properties of the test article.

At the recovery necropsy, the Eckstein Scores of the proximal, mid and distal vagina of females in all groups were all of minimal magnitude, with the exception of findings in the mid vagina of one female in the placebo control group and one female in the 20 mg tamoxifen group that were of mild magnitude. Slight mucification of the epithelium was present in the mid vagina of one female in the 1 mg tamoxifen group and one female in the 20 mg tamoxifen group, indicating lack of complete reversal of this test article-related change over the recovery period. Other microscopic findings in the tamoxifen treated groups were present at a low magnitude with no clear relationship to the test article.

4. Discussion

Tamoxifen is a first generation SERM (Pinkerton and Thomas, 2014; Wardell et al., 2014) that historically has been used in the context of treating breast cancer (Fisher et al., 1998; Fisher et al., 1999; Jordan, 1990). Orally administered tamoxifen has been found to lead to potentially beneficial effects on vaginal epithelium in women being treated for breast cancer. At the same time, there are noted negative effects in some women on long-term tamoxifen therapy for breast cancer (Mortimer et al., 1999). Since orally administered tamoxifen interacts with estrogen receptors throughout the body, these negative effects may not be observed when tamoxifen is administered locally. As noted, a recent pilot study suggests that vaginal tamoxifen may be useful in the treatment of symptoms of VVA (Chollet et al., 2019).

The present study was conducted as part of a preclinical development program to support evaluation of the pharmacokinetics and local tolerance of tamoxifen in post-menopausal women. Prior to conducting human studies, it is common to evaluate the potential vaginal effects in a relevant animal model such as rabbits. It can be challenging to administer a tablet to the rabbit vagina of any appreciable size. Therefore, the components of the tablet formulation were prepared as a granulation (one step short of preparing a compressed tablet). The granulation should behave similarly to a tablet following the initial stages of disintegration in the vagina.

In this study, tamoxifen accounted for approximately 85% or more of the total systemic exposure to tamoxifen, 4-hydroxytamoxifen, and *N*-desmethyltamoxifen following once daily intravaginal administration of 1 to 20 mg tamoxifen for 28 days to female rabbits. These results suggest that tamoxifen administrated vaginally is metabolized less extensively than observed following oral administration in mice, rats, and humans. Tamoxifen is highly metabolized following oral administration in rats and mice (Lien et al., 1991; Robinson et al., 1991) as well as humans (Jin et al., 2005). Given no detectable metabolites during early treatment and at the lower dose, it is likely that tamoxifen is minimally metabolized in the vaginal wall of rabbits. There are no reported studies on the pharmacokinetics or metabolism of tamoxifen in rabbits by any route of administration. Metabolism of tamoxifen is mediated primarily through cytochrome P450 enzymes. The metabolism of tamoxifen to 4-hydroxytamoxifen is through action of CYP2D6 and metabolism to *N*-desmethyltamoxifen by CYP3A4/5 (Desta et al., 2004). While it is unknown if these enzymes are present in vaginal tissue of rabbits they have been measured in human vaginal tissue. Interestingly mRNA CYP2D6 was not found in human vaginal tissues but CYP3A4 and 5 were detected (To et al., 2013). In addition to these metabolites, another tamoxifen metabolite, 4-hydroxy-*N*-desmethyltamoxifen, is the primarily generated by CYP2D6 (Cronin-Fenton et al., 2014). This metabolite is about 100 times the binding efficiency to estrogen receptors and can represent up to 10% of circulating tamoxifen and its metabolites following steady state dosing (Cronin-Fenton et al., 2014). While human studies are needed, it is expected that metabolism of vaginal administered tamoxifen will be different than observed following oral dosing. If results are similar to those observed in rabbits, there should be significantly less overall metabolism and systemic absorption as compared to oral administration.

The impact of tamoxifen administered at either 1 mg or 20 mg led to mild to minimal vaginal irritation on repeat dosing over 28 days. The doses correspond approximately to 0.28 and 5.7 mg/kg. The lower doses used herein is similar to an oral dose (20 mg) in humans. It is unknown what dose might be effective in treating VVA in post-menopausal women, but needs to tested in adequately controlled human clinical studies. It is important to find the lowest dose at which vaginal tamoxifen will produce the desired pharmacologic outcome to avoid potential local effects on the endometrium (Varras et al., 2003). In addition to lowering the dose, the product can be administered into the lower third of the vagina which may minimize uptake of tamoxifen (and any locally generated metabolites) into the uterus (Cicinelli and de Ziegler, 1999; Cicinelli et al., 2004; Cicinelli et al., 2003; Cicnelli et al., 2000). This study suggests that tamoxifen can be delivered intravaginally with little systemic absorption and metabolism.

5. Conclusions

Overall, tamoxifen was absorbed and metabolized following vaginal administration and local irritation was minimal to none at both doses. These data along with other published studies suggest that vaginally delivered tamoxifen may represent a safe and effective treatment for women suffering from VVA who are at risk, suffering from or are breast cancer survivors, where estrogens are contraindicated.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2019.118691.

References

Bertolissi, A., Cartei, G., Turrin, B., Cioschi, B., Rizzi, V., 1998. Behaviour of vaginal epithelial maturation and sex hormone binding globulin in post-menopausal breast cancer patients during the first year of tamoxifen therapy. Cytopathology 9, 263–270.

Boccardo, F., Bruzzi, P., Rubagotti, A., Nicolò, G., Rosso, R., 1981. Estrogen-like action of tamoxifen on vaginal epithelium in breast cancer patients. Oncology 38, 281–285.

Chollet, J., Meyn, L., Mermelstein, F., 2019. Weekly vaginal administration of tamoxifen for three months in postmenopausal women with vulvar and vaginal atrophy: a possible new treatment approach? Clin. Experiment. Obst. Gynecol. 46, 285–288.

Cicinelli, E., de Ziegler, D., 1999. New Hypotheses. Transvaginal progesterone: evidence for a new functional 'portal system' flowing from the vagina to the uterus. Human Reproduct. Update 5, 365–372.

Cicinelli, E., de Ziegler, D., Morgese, S., Bulletti, C., Luisi, D., Schonauer, L.M., 2004. First uterine pass effect is observed when estradiol is placed in the upper but not lower third of the vagina. Fertil. Steril. 81, 1414–1416.

Cicinelli, E., Di Naro, E., De Ziegler, D., Matteo, M., Morgese, S., Galantino, P., Brioschi, P.-A., Schonauer, S., 2003. Placement of the vaginal 17-estradiol tablets in the inner or outer one third of the vagina affects the preferential delivery of 17-estradiol toward the uterus or periurethral areas, thereby modifying efficacy and endometrial safety. Am. J. Obstet. Gynecol. 189, 55–58.

Cicnelli, E., De Ziegler, D., Bulleti, C., Matteo, M.G., Schonauer, L.M., Galantino, P., 2000. Direct transport of progesterone from vagina to uterus. Obstet. Gynecol. 95, 403–406.

Cronin-Fenton, D.P., Damkier, P., Lash, T.L., 2014. Metabolism and transport of tamoxifen in relation to its effectiveness: new perspectives on an ongoing controversy. Future Oncol. 10, 107–122.

Desta, Z., Ward, B.A., Soukhova, N.V., Flockhart, D.A., 2004. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. J. Pharmacol. Exp. Ther. 310, 1062–1075.

Draize, J.H., Woodard, G., Calvery, H.O., 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. 82, 377–390.

EBCTCG, 2005. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365, 1687–1717.

Eckstein, P., Jackson, M.C.N., Millman, N., Sobrero, A.J., 1969. Comparison of vaginal tolerance tests of spericidal preparations in rabbits and monkeys. J. Reprod. Fertil. 20, 85–93.

Ellmén, J., Hakulinen, P., Partanen, A., Hayes, D.F., 2003. Estrogenic effects of toremifene and tamoxifen in postmenopausal breast cancer patients. Breast Cancer Res. Treat. 82, 103–111.

Etienne, M.C., Milano, G., Fischel, J.L., Frenay, M., François, E., Formento, J.L., Gioanni, J., Namer, M., 1989. Tamoxifen metabolism: pharmacokinetic and in vitro study. Br. J. Cancer 60, 30–35.

Ferrazzi, E., Cartei, G., Mattarazzo, R., Fiorentino, M., 1977. Oestrogen-like effect of tamoxifen on vaginal epithelium. Br. Med. J. 1, 1351–1352.

Fisher, B., Costantino, J.P., Wickerham, D.L., Redmond, C.K., Kavanah, M., Cronin, W.M., Vogel, V., Robidoux, A., Dimitrov, N., Atkins, J., Daly, M., Wieand, S., Tan-Chiu, E., Ford, L., Wolmark, N., Breast, o.N.S.A., Investigators, B.P., 1998. Tamoxifen for prevention of breast cancer: report of the national surgical adjuvant breast and bowel project P-1 study. JNCI 90, 1371–1388.

Fisher, B., Dignam, J., Wolmark, N., Wickerham, D.L., Fisher, E.R., Mamounas, E., Smith, R., Begovic, M., Dimitrov, N.V., Margolese, R.G., Kardinal, C.G., Kavanah, M.T., Fehrenbacher, L., Oishi, R.H., 1999. Tamoxifen in treatment of intraductal breast cancer: National Surgical Adjuvant Breast and Bowel Project B-24 randomised controlled trial. Lancet 353, 1993–2000.

Jin, Y., Desta, Z., Stearns, V., Ward, B., Ho, H., Lee, K.-H., Skaar, T., Storniolo, A.M., Li, L., Araba, A., Blanchard, R., Nguyen, A., Ullmer, L., Hayden, J., Lemler, S., Weinshilboum, R.M., Rae, J.M., Hayes, D.F., Flockhart, D.A., 2005. CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer Treatment. JNCI 97, 30–39.

Jordan, V.C., 1990. Long-term adjuvant tamoxifen therapy for breast cancer. Breast Cancer Res. Treat. 15, 125–136.

Lahti, E., Vuopala, S., Kauppila, A., Blanco, G., Ruokonen, A., Laatikainen, T., 1994. Maturation of vaginal and endometrial epithelium in postmenopausal breast cancer patients receiving long-term tamoxifen. Gynecol. Oncol. 55, 410–414.

Lien, E.A., Solheim, E., Lea, O.A., Lundgren, S., Kvinnsland, S., Ueland, P.M., 1989. Distribution of 4-Hydroxy-*N*-desmethyltamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. Cancer Res. 49, 2175–2183.

Lien, E.A., Solheim, E., Ueland, P.M., 1991. Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. Cancer Res. 51, 4837–4844.

Löser, R., Seibel, K., Roos, W., Eppenberger, U., 1985. In vivo and in vitro antiestrogenic action of 3-hydroxytamoxifen, tamoxifen and 4-hydroxytamoxifen. Eur. J. Cancer Clin. Oncol. 21, 985–990.

Love, R.R., Kurtycz, D.F., Dumesic, D.A., Laube, D.W., Yang, Y., 2000. The effects of tamoxifen on the vaginal epithelium in postmenopausal women. J. Women's Health Gender-Based Med. 9, 559–563.

Miodrag, A., Ekelund, P., Burton, R., Castleden, C.M., 1991. Tamoxifen and partial oestrogen agonism in postmenopausal women. Age Ageing 20, 52–54.

Mortimer, J.E., Boucher, L., Baty, J., Knapp, D.L., Ryan, E., Rowland, J.H., 1999. Effect of tamoxifen on sexual functioning in patients with breast cancer. J. Clin. Oncol. 17 1488-1488.

Neven, P., Vernaeve, H., 2000. Guidelines for monitoring patients taking tamoxifen treatment. Drug Saf. 22, 1–11.

Patel, H.K., Bihani, T., 2018. Selective estrogen receptor modulators (SERMs) and selective estrogen receptor degraders (SERDs) in cancer treatment. Pharmacol. Ther. 186, 1–24.

Pinkerton, J.V., Thomas, S., 2014. Use of SERMs for treatment in postmenopausal women. J. Steroid Biochem. Mol. Biol. 142, 142–154.

Robinson, S.P., Langan-Fahey, S.M., Johnson, D.A., Jordan, V.C., 1991. Metabolites, pharmacodynamics, and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient. Drug Metab. Dispos. 19, 36–43.

Shiota, A., Igarashi, T., Kurose, T., Ohno, M., Hando, T., 2002. Reciprocal effects of tamoxifen on hormonal cytology in postmenopausal women. Acta Cytol. 46, 499–506.

To, E.E., Hendrix, C.W., Bumpus, N.N., 2013. Dissimilarities in the metabolism of antiretroviral drugs used in HIV pre-exposure prophylaxis in colon and vagina tissues. Biochem. Pharmacol. 86, 979–990.

Vardy, M.D., Lindsay, R., Scotti, R.J., Mikhail, M., Richart, R.M., Nieves, J., Zion, M., Cosman, F., 2003. Short-term urogenital effects of raloxifene, tamoxifen, and estrogen. Am. J. Obstet. Gynecol. 189, 81–88.

Varras, M., Polyzos, D., Akrivis, C., 2003. Effects of tamoxifen on the human female genital tract: review of the literature. Eur. J. Gynecol. Oncol. 24, 258–268.

Wardell, S.E., Nelson, E.R., McDonnell, D.P., 2014. From empirical to mechanism-based discovery of clinically useful Selective Estrogen Receptor Modulators (SERMs). Steroids 90, 30–38.

Weiss, H., Martell, B., Constantine, G.D., Davis, S.M., Vidal, J.D., Mayer, P.R., Doorbar, M., Friend, D.R., 2019. Pharmacokinetics and tolerability of a novel 17β-estradiol and progesterone intravaginal ring in sheep. J. Pharm. Sci.