Short Communication: Expression of Transporters and Metabolizing Enzymes in the Female Lower Genital Tract: Implications for Microbicide Research

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Abstract

Topical vaginal microbicides have been considered a promising option for preventing the male-to-female sexual transmission of HIV; however, clinical trials to date have not clearly demonstrated robust and reproducible effectiveness results. While multiple approaches may help enhance product effectiveness observed in clinical trials, increasing the drug exposure in lower genital tract tissues is a compelling option, given the difficulty in achieving sufficient drug exposure and positive correlation between tissue exposure and microbicide efficacy. Since many microbicide drug candidates are substrates of transporters and/or metabolizing enzymes, there is emerging interest in improving microbicide exposure and efficacy through local modulation of transporters and enzymes in the female lower genital tract. However, no systematic information on transporter/enzyme expression is available for ectocervical and vaginal tissues of premenopausal women, the genital sites most relevant to microbicide drug delivery. The current study utilized reverse transcriptase polymerase chain reaction (RT-PCR) to examine the mRNA expression profile of 22 transporters and 19 metabolizing enzymes in premenopausal normal human ectocervix and vagina. Efflux and uptake transporters important for antiretroviral drugs, such as P-gp, BCRP, OCT2, and ENT1, were found to be moderately or highly expressed in the lower genital tract as compared to liver. Among the metabolizing enzymes examined, most CYP isoforms were not detected while a number of UGTs such as UGT1A1 were highly expressed. Moderate to high expression of select transporters and enzymes was also observed in mouse cervix and vagina. The implications of this information on microbicide research is also discussed, including microbicide pharmacokinetics, the utilization of the mouse model in microbicide screening, as well as the *in vivo* functional studies of cervicovaginal transporters and enzymes.

VAGINAL MICROBICIDES are vaginally administered drug products used for preventing or significantly reducing the transmission of sexually transmitted infections, including HIV. Microbicide candidates undergoing clinical development currently are antiretroviral drugs that specifically disrupt one or more critical steps in the HIV life cycle, and many of them are marketed drugs for the treatment of acquired immune deficiency syndrome (AIDS), such as the nucleotide reverse transcriptase inhibitor tenofovir. Since the mucosal surface of the female lower genital tract is the initial site of sexually transmitted HIV infection, microbicides administered via the vaginal route have been proposed as a promising approach for the prevention of male-to-female HIV transmission.¹ Despite the effectiveness of these drugs in AIDS treatment, robust and reproducible success in HIV prevention

has not been demonstrated for a vaginal gel containing tenofovir. In the CAPRISA-004 study, the 1% tenofovir vaginal gel reduced HIV-1 incidence by 39%.^{2,3} However, the same tenofovir gel in the VOICE clinical trial was discontinued for futility because the incidence in the placebo gel arm was very similar to that of the active tenofovir arm.³ Enhancement of microbicide product effectiveness is required to provide successful means of protection for women.

While multiple strategies may be helpful in enhancing microbicide effectiveness, increasing the drug exposure in the lower genital tract offers one potential strategy. Cervical and vaginal tissue levels will play a critical role in achieving a pharmacological response for a number of microbicide drug candidates, especially those that must enter cervicovaginal immune cells to take effect, such as tenofovir.^{1,4} Clinical

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pharmacokinetic studies of the 1% tenofovir vaginal gel used in CAPRISA 004 and other trials revealed that the vaginal tissue concentration was 1–2% of that in cervicovaginal fluid after gel application, and only 0.6–10% of the drug in vaginal tissue was converted into the active form tenofovir diphosphate.^{5–7} Large interindividual variability in tissue drug exposure was observed, and the HIV incidence rate in the high-exposure group was several times lower than that in the low-exposure group.⁷ Although the measured and estimated vaginal tissue drug concentrations after microbicide application in all patients were usually much higher than the *in vitro* EC₅₀, it is not clear whether all the host immune cells in the cervicovaginal tissue can be effectively protected at this concentration. Indeed the effective *in vivo* concentration required for microbicide efficacy has yet to be determined.³ In this context, it was proposed that one of the goals of future clinical trials should be to attain the highest tolerable drug concentration in the vagina.^{3,7}

Since most microbicide candidates are substrates of transporters and/or metabolizing enzymes,^{8–10} there is growing interest in understanding the role of transporters and enzymes in controlling the level of drug present in the cells in the lower genital tract. Transporters residing on the cell membrane can efflux or uptake drug molecules, controlling the extent of drug entry into the cell.^{4,8,10} The major efflux transporters are the ATP Binding Cassette (ABC) transporters, while the Solute Carrier (SLC) and Solute Carrier Organic Anion (SLCO) transporters are major transporters responsible for uptake.

The major enzymes involved in antiretroviral drug metabolism include Phase I cytochrome P450s (CYPs) and Phase II UDP-glucuronosyltransferases (UGTs). These two enzyme types are located on the microsome membrane and could affect the residence time of their substrate drugs within the cells. It has been well established that these transporters and enzymes control the absorption and disposition of administered drugs in various tissues, such as liver, kidney, and brain.^{4,8–10} However, further investigations on cervicovaginal transporters and enzymes are hampered by the lack of systematic information on transporter and enzyme expression in the ectocervix and vagina of sexually active premenopausal women, which are the major genital sites for microbicide disposition and action.

The current study aimed to fill this knowledge gap and examined the expression of efflux and uptake transporters, as well as Phase I and II metabolizing enzymes in normal human ectocervix and vagina. The focus was on the most commonly examined transporters and enzymes that are relevant to the metabolism and transport of antiretrovirals and other important drug categories, including 9 ABC transporters and 13 SLC transporters, as well as 9 CYP enzymes and 10 UGT enzymes. The expression in mouse cervix and vagina was also examined in comparison to human. These data are relevant since mice are used in the safety testing of microbicides, especially at the initial stage of product screening.^{11,12} Additionally, it is important to establish an *in vivo* model for the functional study of cervicovaginal transporters and enzymes. To our knowledge, this is the first study to systematically characterize the expression of transporters and enzymes in the lower genital tract of premenopausal women.

Reverse transcriptase polymerase chain reaction (RT-PCR) experiments were performed using cDNA samples from

human and mouse cervicovaginal tissues. All human tissues were obtained from premenopausal women through the Tissue Procurement Facilities at the University of Pittsburgh Medical Center under IRB approved protocols. Human ectocervical tissues were obtained from seven women undergoing hysterectomy for benign conditions. Human vaginal tissues were collected from five women with prolapse. Mouse cervix and vagina were from 8-week-old wild-type CD-1 mice. The RT-PCR was performed using the cDNA extracted from pooled or individual tissues, as described in detail in the legends to Supplementary Figs. S1–S8 (Supplementary Data are available online at www.liebertpub.com/aid).

To quantitate the mRNA level, serially diluted liver cDNA was used as a standard, given the abundant expression of the drug transporters and metabolizing enzymes planned for examination in this study in the liver.¹³ The tissue samples were homogenized using a tissue homogenizer and total RNA was extracted using the TRIZOL reagent (Invitrogen Inc.). The reverse transcription was performed by using the SuperScript III First-strand Synthesis Kit (Invitrogen Inc.), and PCR was performed by using the GoGreen Hot-start mastermix (Promega Inc.). The cDNA transcribed from 50 ng of total RNA was used for all the cervicovaginal tissue samples, while the cDNA from 1, 5, and 25 ng total RNA was used for liver standards. The PCR programs consist of a denaturation at 95°C for 4 min, followed by 28 to 36 cycles of 95°C 30 s, 60°C 30 s, and 72°C 30 s. The cycle number for each gene is shown in the legends to Supplementary Figs. S1–S8. The final extension was set at 72°C for 3 min. The PCR products were then analyzed using agarose gel electrophoresis, and gel pictures were captured by using the ChemiDoc MP imaging system (BioRad Inc.). The common gene names and official gene symbols of the transporters and enzymes, as well as the primer sequences used in the PCR, are summarized in Supplementary Tables S1–S4.

Nine of the most important efflux transporters from the ABC superfamily involved in antiretroviral drug disposition^{4,14–17} were examined and the RT-PCR results are summarized in Table 1. The liver samples had highest expression of MRP2, MRP3, and MRP6, followed by P-gp, BCRP, MRP1, MRP5, MRP7, and MRP4 with a moderate or low expression level. Compared to the liver, P-gp, BCRP, MRP1, MRP4, MRP5, and MRP7 were moderately or highly expressed efflux transporters in human cervicovaginal tissues. Inter-individual differences in the expression level of P-gp, BCRP, MRP1, MRP4, and MRP7 were observed. The expression level of P-gp, BCRP, and MRP3 appeared to be different between epithelium and stroma. The ectocervical MRP1 and MRP4 level appeared to be higher than those in the vagina (Supplementary Fig. S1). Thirteen uptake transporters were examined, including 10 from the SLC superfamily and 3 from the SLCO superfamily. These transporters were shown to be relevant to the pharmacokinetics of antiretroviral drugs.^{4,10,15,18–21} Results are summarized in Table 2. OAT2, OCT2, OCT3, ENT1, OATP-D, and OATP-E are the most highly expressed uptake transporters compared to their expression in liver. The discrepancy in the expression level was observed for OAT2, OCT2, OCT3, ENT1, and OATP-D between epithelium and stroma in the ectocervix. The discrepant expression was also observed for OCT3 and OATP-E between epithelium and stroma in the vagina (Supplementary Fig. S3).

TABLE 1. SUMMARY OF EFFLUX TRANSPORTERS EXPRESSION IN HUMAN AND MOUSE CERVICOVAGINAL TISSUES

	Human transporter level (% of human liver)		Mouse transporter level (% of mouse liver)	
	Human ectocervix	Human vagina	Mouse cervix	Mouse vagina
P-gp (Mdr1a/Mdr1b)	++	++	+++ / +++++	++ / +++++
BCRP (Bcrp)	+++	+++	++	++
MRP1 (Mrp1)	++++	+++	++++	++++
MRP2 (Mrp2)	—	—	—	—
MRP3 (Mrp3)	—	—	++	++
MRP4 (Mrp4)	++++	+++	++++	++++
MRP5 (Mrp5)	++++	++++	++++	++++
MRP6 (Mrp6)	—	—	—	—
MRP7 (Mrp7)	++++	++++	++	++

—, ≤2% or undetectable; +, 2–10%; ++, 10–50%; +++, 50–100%; +++++, >> 100%. The corresponding gel images are shown in Supplementary Figs. S1 and S2.

In general, the efflux and uptake transporter expression profiles found for mouse cervix and vagina overlap those in human tissues. However, differences between the two species have been noted. Among the efflux transporters highly expressed in human cervicovaginal tissues, Mdr1a, Mdr1b, Bcrp, Mrp4, and Mrp5 were also highly expressed in mouse cervix and vagina. In addition, mouse Mrp3 has a higher expression compared to human MRP3, while mouse Mrp7 expression is lower than that in human (Table 1). There appeared to be an interindividual difference in the expression of Mdr1b, Mrp2, Mrp3, Mrp6, and Mrp7, but no profound difference was observed between cervix and vagina for all transporters (Supplementary Fig. S2). When comparing uptake transporter expression, interspecies differences were also observed (Table 2). Compared to the highly expressed uptake transporters in human, mouse Oat2, Oatp-D, and Oatp-E were also abundantly expressed, while Oct2 and Ent1 were expressed at low or undetectable levels. In addition, Oat1, Oat3, and Ent2 were expressed at a high level in the mouse, while the expression of their human orthologs was limited in human tissues. There was no obvious difference between mouse cervix and vagina (Supplementary Fig. S4).

The RT-PCR results for Phase I and II enzymes in human cervicovaginal tissue are summarized in Tables 3 and 4. Most Phase I enzymes relevant to anti-HIV therapy, including the CYP2B, 2C, 2D, and 3A families, plus several others generally important for xenobiotic metabolism, were examined. CYP1A1, 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4 were highly expressed in human liver. However, it should be noted that for some isoforms, such as CYP1A1 and 2B6,²² the liver is not the organ with the highest expression. Relative to the expression found in liver, human ectocervix does not express most isoforms of Phase I enzymes, with the exception of CYP1A1 and 1B1 (Table 3 and Supplementary Fig. S5). On the other hand, CYP3A4, the most important Phase I enzyme involved in xenobiotic metabolism, was not detected in human ectocervix (Table 3). In contrast to Phase I enzymes, many Phase II enzyme isoforms were found to be highly expressed in human ectocervix and vagina, including multiple isoforms in the UGT1A and 2B families as summarized in Table 4. Among the highly expressed enzymes, the examined enzymes have a similar expression level in both vagina and cervix. There was no obvious difference between ectocervix and vagina (Supplementary Fig. S7).

TABLE 2. SUMMARY OF UPTAKE TRANSPORTERS EXPRESSION IN HUMAN AND MOUSE CERVICOVAGINAL TISSUES

	Human transporter level (% of human liver)		Mouse transporter level (% of mouse liver)	
	Human ectocervix	Human vagina	Mouse cervix	Mouse vagina
OAT1 (Oat1)	—	—	++	++
OAT2 (Oat2)	++	++	++	++
OAT3 (Oat3)	—	—	++	++
OCT1 (Oct1)	—	—	—	—
OCT2 (Oct2)	++++	++++	—	—
OCT3 (Oct3)	++	+	++	++
ENT1 (Ent1)	+++	+++	++	++
ENT2 (Ent2)	—	—	++++	++++
ENT3 (Ent3)	—	—	+++	+++
CNT1 (Cnt1)	—	—	—	—
OATP-B (Oatp-B)	—	—	+	+
OATP-D (Oatp-D)	++++	++++	++++	++++
OATP-E (Oatp-E)	++	++	+++	+++

—, ≤2% or undetectable; +, 2–10%; ++, 10–50%; +++, 50–100%; +++++, >> 100%. The corresponding gel images are shown in Supplementary Figs. S3 and S4.

TABLE 3. SUMMARY OF PHASE I ENZYMES EXPRESSION IN HUMAN AND MOUSE CERVICOVAGINAL TISSUES

Human Phase I enzymes	Human Phase I enzyme level (% of human liver)		Mouse Phase I enzyme level (% of mouse liver)	
	Human ectocervix	Mouse Phase I enzymes	Mouse cervix	Mouse vagina
CYP 1A1	+++	Cyp1a1	++	++
CYP 1A2	—	Cyp1a2	—	—
CYP 1B1	+++	Cyp1b1	++	++
CYP 2C8	+	Cyp2b10	+	—
CYP 2C19	—	Cyp2c29	—	—
CYP 2B6	—	Cyp2c40	+	—
CYP 2C9	—	Cyp3a11	+	—
CYP 2E1	—			
CYP3A4	—			

—, ≤ 2% or undetectable; +, 2–10%; ++, 10–50%; +++, 50–100%; + + + +, > 100%. The corresponding gel images are shown in Supplementary Figs. S5 and S6.

The expression of mouse enzymes that are orthologous to human CYP1A1, CYP1B1, and UGT1A1 was examined in cervix and vagina and summarized in Tables 3 and 4. Mouse Cyp1a1, Cyp1b1, and Ugt1a1 are moderately expressed as compared to the mouse liver levels. In addition to these three enzymes, several other commonly studied Cyp and Ugt isoforms were also examined in mouse cervicovaginal tissue. Ugt1a2 and 1a6 were found to be highly expressed. No profound difference between mouse cervix and vagina was observed in terms of expression level (Supplementary Figs. S6 and S8).

The mRNA expression profiles of transporters and enzymes in liver and cervicovaginal tissues obtained in the present study are generally consistent with published studies. In human liver, it was reported that efflux and uptake transporters including P-gp, BCRP, MRP2, MRP3, MRP6, OAT2, OCT1, OCT3, ENT1, and CNT1 were moderately to highly expressed, as examined by using microarray and real-time PCR assays.^{13,23} Bieche *et al.* showed that human liver had moderate to high levels of mRNA expression of CYP enzymes including CYP2C8, 2C9, 2E1, and 3A4, by using real-time PCR.²² In addition, multiple UGT isoforms in the UGT1A and 2B families were reported to be highly expressed in human liver, as detected by using RT-PCR.²⁴ Evident mRNA expression of these transporters and enzymes in human liver tissue was also observed in this study.

As to the expression in cervicovaginal tissues, there have been studies that reported findings similar to the present study. A microarray study examined 50 xenobiotic transporters in human tissues and suggested that a number of efflux and uptake transporters were expressed at a high percentile among all the genes expressed in the cervix.²³ Schneider *et al.* reported that P-gp was abundantly expressed within normal cervical epithelium by using immunohistochemical (IHC) staining.²⁵ Maliepaard *et al.* found that the BCRP mRNA level was high among all the tissues tested including liver and small intestine by using real-time PCR.²⁶ In this study the intensive protein expression of BCRP was also detected in the cervix by IHC staining, and the strongest signal was concentrated on the venous endothelium.²⁶ Farin *et al.* reported that CYP1A1 was highly expressed in human cervical epithelial cell culture using RT-PCR and IHC staining approaches.²⁷ Previous IHC staining work by Yokose *et al.* and Patel *et al.* showed undetectable expression of CYP3A4 in normal cervix and vagina.^{28,29}

In line with these published studies, our study revealed that a number of transporters and metabolizing enzymes that can potentially contribute to microbicide drug exposure patterns were expressed in the human female lower genital tract. Six efflux transporters (P-gp, BCRP, MRP1, 4, 5, and 7) and six uptake transporters (OAT2, OCT2, OCT3, ENT1, OATP-D,

TABLE 4. SUMMARY OF PHASE II ENZYMES EXPRESSION IN HUMAN AND MOUSE CERVICOVAGINAL TISSUES

Human Phase II enzymes	Human Phase II enzyme level (% of human liver)		Mouse Phase II enzymes	Mouse Phase II enzyme level (% of mouse liver)	
	Human ectocervix	Human vagina		Mouse cervix	Mouse vagina
UGT1A1	+++	+++	Ugt 1a1	++	++
UGT1A3	—	—	Ugt 1a2	++	++
UGT1A4	++	++	Ugt 1a6	+++	+++
UGT1A7	++++	++++	Ugt 1a9	+	+
UGT1A8	+++	+++	Ugt 2b5	—	—
UGT1A10	+++	+++			
UGT2B4	+++	+++			
UGT2B7	—	—			
UGT2B15	+++	+++			
UGT2B17	+++	+++			

—, ≤2% or undetectable; +, 2–10%; ++, 10–50%; +++, 50–100%; + + + +, > 100%. The corresponding gel images are shown in Supplementary Figs. S7 and S8.

TABLE 5. ANTIRETROVIRAL DRUGS THAT COULD BE AFFECTED BY THE HIGHLY EXPRESSED TRANSPORTERS AND METABOLIZING ENZYMES IN HUMAN CERVICOVAGINAL TISSUES

Superfamily	Name of transporter/enzyme	Expression level in human ectocervix/vagina	Antiretroviral drugs as substrates	Drug class
ABC transporters (efflux)	P-gp	++/++	Maraviroc, raltegravir, amprenavir, nelfinavir, atazanavir, ritonavir, lopinavir, saquinavir, darunavir, indinavir, tipranavir, nelfinavir, tenofovir DF, abacavir	EI, RTI, PI, II
	BCRP	+++ / +++	Zidovudine, efavirenz, atazanavir, lamivudine, stavudine, didanosine, abacavir	RTI, PI
	MRP4	+++ / +++	Tenofovir, zidovudine, abacavir	RTI, PI
	MRP5	+++ / +++	Zidovudine, stavudine	RTI
	MRP7	+++ / +++	Tenofovir, nevirapine	RTI
SLC transporters (uptake)	OAT2	++/++	Azidothymidine	RTI
	OCT2	+++ / +++	Lamivudine, zalcitabine	RTI
	OCT3	++/+	Lamivudine	RTI
	ENT1	+++ / +++	Didanosine, zalcitabine	RTI
Phase I CYP enzymes	CYP1A1	+++	Affects the effectiveness of HAART in female smokers	RTI, PI
Phase II UGT enzymes	UGT1A1	+++ / +++	Efavirenz, raltegravir, elvitegravir, dolutegravir	RTI, II

Expression levels in ectocervix/vagina were presented as % of those in human liver, and the data were extracted from Tables 1–14. —, ≤2% or undetectable; +, 2–10%; ++, 10–50%; +++, 50–100%; +++++, >100%. EI, entry inhibitor; RTI, reverse transcriptase inhibitor; PI, protease inhibitor; II, integrase inhibitor.

and OATP-E), as well as two CYPs (CYP1A1 and 1B1) and seven UGTs (UGT 1A1, 1A7, 1A8, 1A10, 2B4, 2B15, and 2B17), were found to be highly expressed. On the other hand, some transporters and enzymes isoforms that are commonly detected in metabolic organs, such as the CYP2D, 2E, and 3A families, were expressed at low level or were even undetectable in the female lower genital tract. Overall, our observations in the present study are consistent with the published data in human cervix and vagina.

The highly expressed transporters and enzymes in the lower genital tract may have an impact on the cervicovaginal exposure of various microbicide candidates as summarized in Table 5. This list of drugs is summarized based on existing reviews.^{4,8–10} In the current drug discovery and development programs, the study of the potential effect of transporters and enzymes on drug candidates is usually confined to a select panel of the most predominant transporters and enzymes, such as P-gp and CYP3A4. For antiretroviral drug metabolism studies involving Phase II UGT enzymes, most studies were focused on UGT1A1 and 2B7, while the metabolism mediated by other highly expressed UGTs in cervicovaginal tissue such as UGT1A7, 1A10, 2B15, and 2B17 were poorly characterized. It is possible that as the antiretroviral drugs will be subjected to more thorough screening, more microbicide candidates will be included in this table. Of note, some transporters and enzymes isoforms may impact microbicide PK-PD indirectly. For example, CYP1A1 is not directly involved in the inactivation of antiretrovirals, however, the CYP1A1 genotype was reported to impact the treatment outcome of highly active antiretroviral therapy (HAART) among female smokers.³⁰ The genotype with higher activity is associated with impaired HAART effectiveness. Since this enzyme could convert smoke-derived compounds to DNA-damaging agents that promote HIV-1 gene expression and replication, the proposed explanation is that higher CYP1A1 activity contributes to a

higher concentration of DNA-damaging agents in HIV-infected tissues.³⁰ Whether the cervicovaginal CYP1A1 could impact the microbicide efficacy among female smokers would be an interesting topic for future research.

The mRNA expression of transporters and enzymes does not necessarily correlate with their activity in microbicide absorption and disposition, and further work is needed to confirm the functional activity of the transporters and enzymes that are moderately or highly expressed. Considering the number of transporters and enzymes that showed high expression in cervicovaginal tissue, information on protein localization and mRNA/protein regulation will facilitate the prioritization and experimental design of the functional studies of transporters and enzymes. The information on the localization will help predict the specific role of transporters and enzymes in microbicide pharmacokinetics.

The transporters and enzymes localized in the epithelium, for example, P-gp,²⁵ may be able to limit the lumen-to-tissue drug distribution and tissue-to-lumen drug efflux, and therefore may be relevant to the pharmacokinetics of drugs administered via both vaginal and systemic routes. The transporters and enzymes localized within the venous endothelium such as BCRP may exert unidirectional control on blood-to-tissue drug distribution while not affecting lumen-to-tissue drug penetration. In addition, the localization information will help rationally select the model to study the functionality of transporters and enzymes. As mentioned above, a transporter or enzyme could be located in different kinds of cells, such as epithelial cells and venous endothelial cells. If a transporter/enzyme is exclusively located in one type of cell, e.g., endothelial cell, then the impact of the modulation of transporters and enzymes on microbicide pharmacokinetics could be studied using primary or immortalized cell culture that reconstitutes the specific cell type *in vitro*. However, if a transporter/enzyme is located in

multiple cell types, then a clinically relevant animal model should be used to provide comprehensive understanding of the *in vivo* effect of the modulation of transporters and enzymes on the cervicovaginal tissue exposure of substrates. This *in vivo* model must possess intact cervicovaginal tissues that are anatomically and physiologically comparable to the human female genital tract.

Besides protein localization, the understanding of the factors that can regulate the expression of transporters and enzymes and activity in cervicovaginal tissues will also aid in the functional study. As reported in other tissues, the transporters and enzymes that are highly expressed in vagina and cervix are subject to complex regulation mechanisms. One example is the effect of sex hormones on the expression and activity of transporters and enzymes.³¹ Since sex hormones are deposited in the lower genital tract and their concentrations can be affected by various factors (race, age, menstrual stage, and contraception choice),³² it is possible that the expression and activity of transporters and enzymes will change in different scenarios, causing interindividual and intraindividual variability in cervicovaginal drug concentration and efficacy. It is therefore prudent to identify the scenarios that transporters and enzymes have differential expression/activity compared to the basal status, and understand whether this will result in a difference in the pharmacokinetic profile and efficacy of administered drugs that target the lower genital tract.

The comparison in expression patterns of cervicovaginal transporters and enzymes between human and mouse has implications for the utilization of a mouse model in the microbicide testing and functionality study of cervicovaginal transporters and enzymes. Although nonhuman primates are considered as the most clinically relevant animal model to study STI transmission and prevention, the wild-type mouse is also used in safety evaluations as a convenient *in vivo* model.^{11,33, 34} It has been shown that the toxicity profile of a number of microbicide candidates in progesterone synchronized mice correlated well with clinical trial results.^{12,35,36} Nevertheless, our finding suggests that caution is needed when interpreting data from mouse studies.

Since drug toxicity is dependent on the tissue exposure of the drug, it is important to understand the avidity of the microbicide candidate to the transporters and enzymes for proper prediction of human safety. In addition, the interspecies difference in the expression of cervicovaginal transporters and enzymes suggests that caution should be implemented when using mouse as the model for the *in vivo* functional study of cervicovaginal transporters and enzymes. The expression pattern in mouse cervix and vagina overlaps with that of human but is not identical. To study the *in vivo* role of transporters and enzymes in cervicovaginal drug disposition, the measurement of cervicovaginal tissue concentration is required. It seems appropriate to start with a convenient animal model.

The common strategy of functional study of one transporter/enzyme is to examine whether the probe drug disposition changes in response to the modulation of that transporter/enzyme isoform. Many probe substrates and modulators are affected or act on more than one transporter/enzyme isoform, so that the selection of probe and modulator should be as specific as possible if the mouse model is to be used. More than one type of probe and/or modulator may be

needed to address the interspecies difference in transporter and enzyme expression. For example, the effect of MRP4 inhibition on substrate disposition could be studied using tenofovir as the probe substrate. Tenofovir is an avid substrate of MRP4, but it is also transported by the uptake transporters OAT1 and OAT3.^{4,8,10} The human lower genital tract has only MRP4 expression whereas mouse has all of the three transporters highly expressed in the cervix and vagina.

When using mouse as a model, caution is needed to ensure that the MRP4 inhibitor does not affect OAT1 or OAT3 activity, otherwise the conclusion obtained in mouse may significantly deviate from clinical trial results. Thus, the expression profiles in mouse cervix and vagina are valuable information for the mouse studies that involve cervicovaginal transporters and enzymes. It should be noted that the difference in mRNA does not necessarily reflect a difference in activity. Furthermore, mice express different amounts of transporter and enzyme isoforms compared to human (e.g., 103 CYP genes in mice versus 57 in humans), and it is possible that enzymes involved in the metabolism of an antiretroviral in humans would belong to a completely different subfamily in mice.

Further work is needed to determine whether the interspecies difference occurs at all levels (mRNA, protein, activity), and whether the transport of important antiretroviral drugs is species-dependent. In addition to mouse, the macaques and rabbits are considered as more clinically relevant models in microbicide research, and are required by FDA for the efficacy and safety testing of microbicides. The expression of multiple transporters and enzymes in human and mouse cervicovaginal tissues warrants further investigation on cervicovaginal transporters and enzymes in those higher species.

To summarize, this study is the first to provide a systematic evaluation of the expression profile of transporters and enzymes for the ectocervix and vagina of premenopausal women. The information on mRNA expression of transporters and enzymes in the lower genital tract will facilitate the understanding of the role of transporters and enzymes in microbicide pharmacokinetics, and will likely contribute to the development of novel strategies aimed for achieving adequate tissue levels of microbicide products. This study also provides critical information that may be utilized for rational experimental design and data interpretation for mouse models utilized in microbicide screening and for *in vivo* functional study of cervicovaginal transporters and enzymes. Nevertheless, this study is not without its limitations. The conventional RT-PCR approach used in this study may not be as accurate as other quantitative approaches, such as real-time RT-PCR. In addition, mRNA level does not necessarily correlate with protein level and transport/enzyme activity. Therefore, further validation of the observed transporter expression profiles at multiple levels (mRNA, protein, activity/functionality) appears necessary for future research in cervicovaginal transporters and metabolizing enzymes.

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