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Pharmacokinetic and Pharmacodynamic Evaluation following Vaginal Application of IQB3002, a Dual-Chamber Microbicide Gel Containing the Nonnucleoside Reverse Transcriptase Inhibitor IQP-0528 in Rhesus Macaques

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We evaluated the *in vivo* pharmacokinetics and used a complementary *ex vivo* coculture assay to determine the pharmacodynamics of IQB3002 gel containing 1% IQP-0528, a nonnucleoside reverse transcriptase inhibitor (NNRTI), in rhesus macaques (RM). The gel (1.5 ml) was applied vaginally to 6 simian-human immunodeficiency (SHIV)-positive female RM. Blood, vaginal fluids, and rectal fluids were collected at 0, 1, 2, and 4 h. RM were euthanized at 4 h, and vaginal, cervical, rectal, and regional lymph node tissues were harvested. Anti-human immunodeficiency virus (HIV) activity was evaluated *ex vivo* by coculturing fresh or frozen vaginal tissues with activated human peripheral blood mononuclear cells (PBMCs) and measuring the p24 levels for 10 days after an HIV-1_{Ba-L} challenge. The median levels of IQP-0528, determined using liquid chromatography-tandem mass spectroscopy (LC-MS/MS) methods, were between 10^4 and 10^5 ng/g in vaginal and cervical tissue, between 10^3 and 10^4 ng/g in rectal tissues, and between 10^5 and 10^7 ng/ml in vaginal fluids over the 4-h period. The vaginal tissues protected the cocultured PBMCs from HIV-1 infection *ex vivo*, with a viral inhibition range of 81 to 100% in fresh and frozen tissues that were proximal, medial, and distal relative to the cervix. No viral inhibition was detected in untreated baseline tissues. Collectively, the median drug levels observed were 5 to 7 logs higher than the *in vitro* 50% effective concentration (EC_{50}) range (0.21 ng/ml to 1.29 ng/ml), suggesting that 1.5 ml of the gel delivers IQP-0528 throughout the RM vaginal compartment at levels that are highly inhibitory to HIV-1. Importantly, antiviral activity was observed in both fresh and frozen vaginal tissues, broadening the scope of the *ex vivo* coculture model for future NNRTI efficacy studies.

HIV-1 microbicide clinical trials have primarily been focused on vaginal gels as a topical preexposure prophylaxis (PrEP) modality to prevent virus acquisition through vaginal intercourse in at-risk women. However, several behavioral studies and clinical trial reports have shown that women also engage in unprotected receptive anal intercourse (RAI), with the highest rates of RAI being found among female sex workers and patients at sexually transmitted infection clinics (1–9). In addition, a recent meta-analysis demonstrated that the estimated per-act human immunodeficiency (HIV) transmission risk (per 10,000 exposures) for RAI is 138, compared to 11, 8, and 4 for insertive anal intercourse, receptive penile-vaginal intercourse, and insertive penile-vaginal intercourse, respectively (10). The risk of HIV acquisition via unprotected RAI may be further exacerbated through the improper use of vaginal microbicide gels or high osmolality personal lubricants in the rectal compartment, which have been shown to cause cellular inflammation and epithelial damage (11–19). These studies collectively highlight a need for a microbicide formulation that is specifically designed for safe application in both the vaginal and rectal compartments and that may also serve as a lubricant. From a user acceptability perspective, a single product for both compartments is more convenient and cost-effective than two separate products. To achieve this, previously developed vaginal microbicide gels (20, 21) were modified to produce a dual-chamber (vaginal and/or rectal application) microbicide gel, IQB3002, which is compatible with the release and stability of the active pharmaceutical ingredient (API), IQP-0528 (22). The IQP-0528 compound has a high therapeutic index (>1 million) (23), and the IQB3002 formulation has been shown to have no detectable cytotoxicity to vaginal and rectal cell lines and no toxic effects on the integrity and viability of ectocervical and colorectal tissue explants over a 24-h exposure period (22). Normal vaginal microflora (lactobacilli) were also shown to be unaffected (22). IQP-0528 is a small molecule pyrimidinedione that potently inhibits the replication of a wide range of laboratory and clinical isolates of HIV (24–26) and has been shown to have a low 90% inhibitory concentration (IC_{90}) in both cell-based and explant tissue models (0.43 μM [146 ng/ml] in JT-CCR5 cells treated with IQP-0528 *in vitro*; 10.1 μM

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[3,440 ng/ml] in JT-CCR5 cells cocultured with human ectocervical explant tissue exposed to IQP-0528 *ex vivo*) (27). Unlike tenofovir (TFV) diphosphate, the active anabolite of the nucleoside reverse transcriptase inhibitor (NRTI) TFV and its prodrug forms, IQP-0528 is not confined to the intracellular compartment and can diffuse freely in and out of the cellular environment in its active state. This property allows for high dosing of tissues with free drug that is readily available to cells trafficking through the tissues. In addition, we have previously shown that a TFV-IQP-0528 combination gel designed for vaginal-only application exhibited favorable safety and efficacy profiles when tested on ectocervical and colorectal tissues *ex vivo* (28). However, no studies to date have evaluated dual-compartment gels *in vivo*.

Macaque models have long been the gold standard for *in vivo* microbicide gel testing, and studies thus far have typically employed up to 4- to 5-ml gel volumes for vaginal dosing in rhesus macaques (RM) (29–36). However, given that the volume of gel recommended for use by women in microbicide clinical trials is also 4 ml, there is a concern that a near-equivalent volume in comparatively smaller macaque models is excessive, which might potentially affect the relevance of drawing parallels between preclinical animal model studies and clinical trials with humans. The surface area of the female RM vaginal tract is approximately 4- to 6-fold smaller than that of women (D. Katz, personal communication). Thus, based on these measurements, a reduced vaginal dose of IQB3002 gel was used in this study, with the objective of performing a pharmacokinetic (PK) analysis and an in-depth comparative pharmacodynamic (PD) evaluation of fresh and frozen vaginal tissue specimens collected after gel application in female RM.

We have previously described a coculture model that measures the ability of genital tract tissues, either treated or not treated with the API, to protect *ex vivo* cocultured T cells and peripheral blood mononuclear cells (PBMCs) from infection with CCR5-tropic HIV-1 (27). The coculture assay is a valuable method to evaluate the efficacy of a microbicide product *ex vivo* and to draw PK/PD correlations, which help inform further development and optimization of the test product prior to expensive scaled-up manufacturing and complex efficacy trials. However, the logistical constraints of early phase I/II microbicide safety trials limit the collection and/or transport of fresh viable tissue samples for PD analysis, and laboratories often have to rely on frozen samples for downstream assessment of tissue susceptibility to a virus challenge. The ability of HIV to infect fresh versus previously frozen tissue has not been well documented, but a study using colorectal tissue showed that freeze-thawing samples is problematic (37). In addition to providing a unique opportunity to collect and evaluate both fresh and previously frozen samples in parallel, macaque models enable validation of the coculture model. The results generated from the current PK/PD study of IQB3002 gel containing 1% IQP-0528 will inform and benefit future nonnucleoside reverse transcriptase inhibitor (NNRTI) trials that rely heavily on frozen specimens for PD analysis.

MATERIALS AND METHODS

Drug and product formulation. IQB3002 gel was manufactured by ImQuest BioSciences (Frederick, MD). The gel contains 1% (wt/wt) IQP-0528 (24–26), 25 mM phosphate buffer (93.9% [wt/wt]), glycerol (2.5% [wt/wt]), methyl paraben (0.2% [wt/wt]), propyl paraben (0.05% [wt/wt]), hydroxyethyl cellulose (2.1% [wt/wt]), and Carbopol [poly(acrylic acid) 0.25% (wt/wt)] (22). It has a pH of 6.08, osmolality of 260 mosmol/kg, and viscosity at 1 s^{-1} of 89.79 ± 5.97 Pa · s. The release rate of IQP-0528 from the gel is 22.85 ± 0.19 μg/cm^3 h with an absorption of 65.89 ± 8.332 (22). The product was stored at room temperature (25°C) in a moisture-free environment until use.

TABLE 1 Specimen collections from female rhesus macaques for PK and PD analysis[a]

Sample	Collection at time point:				
	Prenecropsy[b]	0 min[c]	1 h	2 h	4 h
Blood[d]		X	X	X	X
Vaginal spear/swab[e]		X	X	X	X
Vaginal biopsy[b]	X				
Rectal spear[f]		X	X	X	X
CVL fluid[g]		X			X
Vaginal pH[h]		X	X	X	X
Necropsy[i]					X

[a] X indicates that a sample was collected from rhesus macaques ($n = 6$).
[b] Punch biopsy samples at proximal, medial, and distal sites relative to the cervix were collected to determine drug PD at baseline using the coculture model.
[c] Samples were collected immediately prior to 1.5 ml IQB3002 application.
[d] For blood samples, drug level measurements were performed.
[e] For vaginal spears, drug level measurements were performed. Samples were collected at proximal and distal sites relative to the cervix.
[f] For rectal spears, drug level measurements were performed.
[g] CVL fluid samples (5 ml) were collected for drug level measurements.
[h] For safety assessment.
[i] At necropsy, blood and vaginal fluids were collected for drug level measurements. Vaginal, cervical, and rectal tissues and inguinal and iliac lymph node tissues were also collected. Tissues were used for drug level measurements and/or coculture model experiments for drug PD assessment.

RM and study design. Six sexually mature simian-human immunodeficiency (SHIV)-positive female RM with an average body mass of 5 kg were utilized in this study. All RM were housed under approved biosafety level 2 containment conditions at the Centers for Disease Control and Prevention (CDC), and their diet, care, and maintenance conformed to the *Guide for the Care and Use of Laboratory Animals* (38). All procedures outlined in this study were approved by the Centers for Disease Control and Prevention Institutional Animal Care and Use Committee (IACUC). All RM were placed under appropriate anesthesia prior to any procedures using a 10-mg/kg ketamine mixture intramuscularly or another appropriate anesthetic such as Telazol (2 to 6 mg/kg intramuscularly), as determined by the CDC Animal Resources Branch (ARB) standard operating protocol and ARB staff, including the attending veterinarian. Following anesthesia, each RM received 1.5 ml of IQB3002 which was delivered into the posterior vagina, near the cervix, using a sterile 10-ml syringe attached to a sterile gastric feeding tube (size, 5 or 9 French; length, 7 to 8 cm). The animals were maintained recumbent under anesthesia with the pelvis slightly elevated for 30 min to minimize gel leakage. All RM were euthanized at the 4-h time point. The euthanasia solution (100 mg/kg pentobarbital) is given intravenously according to the CDC ARB standard operating protocol and under direct supervision of the attending veterinarian and/or trained ARB staff. Humane methods of euthanasia with species-appropriate methods that are in accordance with the 2013 American Veterinary Medical Association (AVMA) Guidelines on Euthanasia (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf) are always used.

Specimen collections. Specimens for PK and PD assessments, including blood plasma, cervicovaginal lavage (CVL) fluid, vaginal punch biopsy specimens, and vaginal secretions, were collected at baseline and immediately prior to (0 h) and at specific time intervals (0.5 h, 1 h, 2 h, and 4 h) after IQB3002 application (Table 1). Vaginal, cervical, rectal, and regional (iliac and inguinal) lymph node tissues were collected at necropsy following euthanasia. Blood was collected in

cell preparation tubes (BD Biosciences) and processed using standard Hypaque-Ficoll separation to isolate peripheral blood mononuclear cells (PBMCs) and plasma. Vaginal secretions were collected proximal (near the ectocervix, at the site of product application) and distal (near the introitus) to the product application area by using preweighed Weck-Cel cellulose spears (Medtronic, Minneapolis, MN) or preweighed sponges fitted into a multiswab device (39). Similarly, rectal fluids were collected using preweighed Weck-Cel spears. All spears/sponges and CVL fluid samples were monitored for the presence of blood or discoloration. The CVL fluid collection involved the gentle infusion of 4 to 5 ml of phosphate-buffered saline solution into the vaginal vault by using a sterile 10-ml syringe attached to a sterile gastric feeding tube (size, 5 or 8 French) of adjusted length, and the CVL fluid was drawn out with the same device. The vaginal pH was determined by collecting a swab of vaginal fluid prior to the CVL fluid and rolling it on a pH colorimetric indicator strip (Millipore). Vaginal punch biopsy specimens (between 10 and 50 mg) were collected at baseline from two RM using a rotating biopsy punch tip (Cooper Surgical, Inc., Trumbull, CT) from proximal, medial (midvaginal), and distal sites relative to the cervix in the vaginal compartment. Each biopsy sample was placed in a sterile cryovial, wrapped in foil, and snap-frozen, followed by storage at −80°C until drug PK analysis. For PD analysis, biopsy samples either were kept fresh in 200 μl of RPMI medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U penicillin G/ml, and 100 μg/ml streptomycin sulfate (Gibco) (complete RPMI medium) and transported to the Albert Einstein College of Medicine lab at 4°C overnight or were snap-frozen and stored at −80°C prior to use in the PD coculture assay. Vaginal, cervical, rectal, and lymph node tissues that were collected at necropsy were sectioned, weighed (between 0.1 and 0.9 g), snap-frozen, and stored at −80°C until drug analysis. The vaginal tissue secretions were either kept fresh or were frozen prior to use in the PD coculture assay.

Measurement of IQP-0528 concentrations. Vaginal fluids in individual Weck-Cel spears and multiswab sponges (2 proximal and 2 distal per animal per time point) and rectal fluids in individual Weck-Cel spears (2 spears per animal per time point) were analyzed for IQP-0528 levels. For tissues collected at necropsy from an RM, 3 sections from vaginal (1 proximal, 1 medial, and 1 distal per animal) and 3 sections from cervical and rectal tissues were each analyzed. Given that IQP-0528 is not retained intracellularly and is rapidly transported in and out of cells, tissues were not washed prior to drug analysis or before use in the coculture assay described below, as this would wash out the drug (27). For plasma, two 500-μl aliquots per animal per time point were analyzed. Similarly, 1-ml aliquots of CVL fluid per animal per time point were analyzed for drug levels. The concentrations of IQP-0528 in the biological samples were determined based on a previously described liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (40) and is detailed in our previous study (41). Validation analysis revealed no significant effect of a single freeze-thaw cycle on the IQP-0528 concentrations in plasma, Weck-Cel spears, and multiswab sponge samples (data not shown). The lower limit of quantitation (LLOQ) was defined as the minimum level at which quantitative results could be obtained and was calculated to be 10 times the standard deviation of an injection at the lowest concentration which was statistically different from that for a blank injection using a 99% confidence interval. The LLOQ of IQP-0528 for tissues, vaginal or rectal fluids, and plasma was determined to be 10 ng/sample and 10 ng/ml, where applicable. The amount of vaginal fluid absorbed onto the wick or sponge was measured by weighing before and after fluid absorption. All necropsy tissue samples were also weighed after collection. To convert weight/weight concentrations of IQP-0528 (nanograms per gram of fluid or tissue) to molarity (micromolar), vaginal fluid and tissue densities of 1.0 g/ml were assumed.

Coculture model for evaluation of drug PD. Fresh and frozen biopsy samples were minced into fragments smaller than 1 mm and cocultured with activated human PBMCs from a single donor. Cocultures of fresh and frozen tissues were added to triplicate wells of round-bottomed 96-well plates in the presence of the culture medium used during transport or complete RPMI medium, respectively. Cocultures were incubated at 37°C for 72 h to allow released drug to be transferred from the tissue to the target cells prior to challenge with HIV-$1_{Ba\text{-}L}$ (approximately 10^3 50% tissue culture infective dose [$TCID_{50}$]) as described previously (27). The inoculum was removed, and cocultured tissue and cells were washed three times with serum-free medium. HIV replication was assessed by measuring p24 antigen in culture supernatants 4, 7, and 10 days postchallenge (HIV p24 AlphaLISA kit; PerkinElmer); analysis is shown for day 10 only as background and variable levels were observed on days 4 and 7.

Statistical analyses. Drug level and viral inhibition data are shown as median values unless indicated otherwise. To compare the statistical differences in drug levels across multiple time points, a one-way analysis of variance (ANOVA) was applied. The differences in viral inhibition between the baseline control and postdosing cocultures were compared by Mann-Whitney tests. Analyses were performed using GraphPad Prism (GraphPad Software, Inc.). A P value of <0.05 was considered significant.

RESULTS

Pharmacokinetic analysis. A volume of 1.5 ml of IQB3002 gel was chosen by reducing the volume recommended for use by women enrolled in clinical trials of HIV microbicide gel products, based on the approximate surface area of the RM vaginal compartment. The PK analysis of tissues collected at necropsy (4 h) showed median concentrations of 1.88×10^4 ng/g (55.2 μM) and 2.37×10^4 ng/g (69.6 μM) in vaginal and cervical tissues, respectively (Table 2), with median concentrations being in the range of 10^4 to 10^5 ng/g in proximal, medial, and distal vaginal tissues (Fig. 1A). Notably, vaginal dosing of 1.5 ml of IQB3002 gel also resulted in detectable levels of IQP-0528 in rectal tissues that were collected at 4 h, with a median concentration of 1.76×10^3 ng/g (5.17 μM) being observed. The median concentrations of IQP-0528 in vaginal fluids collected from Weck-Cel spears and multiswab sponges at both the proximal and distal sites relative to the cervix 1 h after vaginal dosing with 1.5 ml of IQB3002 gel were on the orders of magnitude of 10^5 to 10^6 ng/ml (Fig. 1B and Table 2). While a statistically significant ($P < 0.05$) decline in IQP-0528 levels (1.52×10^6 to 1.3×10^5 ng/ml) was observed in vaginal fluids at sites distal to the cervix over the 4-h period (Table 2 and Fig. 1B), the median vaginal fluid drug levels still remained at $>10^5$ ng/ml at both the proximal and distal sites up to 4 h after gel application. The concentrations of IQP-0528 detected in CVL fluid at 4 h after gel application were on the orders of magnitude of 10^4 to 10^5 ng/ml (Fig. 1B), which is consistent with the 10-fold dilution factor of CVL fluid relative to that of vaginal fluid collected directly with vaginal wicks. The *in vitro* 50% effective concentrations (EC_{50}s) for IQP-0528 in the IQB3002 gel formulation are indicated in Fig. 1. Systemic drug absorption was not apparent after vaginal application of the IQB3002 gel, as the IQP-0528 levels were below the LLOQ (10 ng/ml) in blood plasma (data not shown). Similarly, the analysis of rectal fluids and regional lymph node tissues showed drug levels as being below the LLOQ (data not shown).

Pharmacodynamic analysis in fresh and frozen genital tract tissues. The animals were euthanized 4 h after DuoGel application. At necropsy, genital tract tissues were collected 4 h postdosing with IQB3002 gel to assess the ability of drug present in tissue biopsy specimens to protect cocultured HIV target cells from challenge. In addition, we were able to directly compare the ability of fresh versus frozen tissues to provide IQP-0528 to cocultured target cells by determining the susceptibility to an HIV challenge.

TABLE 2 Concentrations of IQP-0528 in tissues and vaginal fluids from rhesus macaques following vaginal application of 1.5 ml of IQB3002[a]

Value type	Vaginal fluid concn, ng/ml (μM) at:						Tissue conc (ng/g ([μM]) at 4 h:			
	1 h		2 h		4 h					
	Proximal	Distal	Proximal	Distal	Proximal	Distal	Vaginal[b]	Cervical	Rectal	
Median	1.54×10^6 (4,509)	1.52×10^6 (4,468)	1.08×10^6 (3,176)	3.17×10^5 (931)	7.81×10^5 (2,296)	1.30×10^5 (382)	1.88×10^4 (55.2)	2.37×10^4 (69.6)	1.76×10^3 (5.17)	
Minimum	2.71×10^5 (797)	6.95×10^5 (2,042)	3.70×10^5 (1,087)	1.54×10^4 (45.1)	3.95×10^5 (1,159)	3.11×10^4 (91.4)	2.00×10^2 (0.59)	1.97×10^3 (5.79)	5.69×10^2 (1.65)	
Maximum	1.86×10^6 (5,454)	4.99×10^6 (14,665)	2.88×10^6 (8,460)	1.82×10^6 (5,352)	1.92×10^6 (5,627)	2.95×10^5 (867)	1.33×10^5 (391)	2.56×10^5 (752)	6.05×10^3 (17.8)	

[a] The medians and ranges of the drug levels detected from rhesus macaques ($n = 6$) are shown.
[b] The values shown are from proximal, medial, and distal vaginal tissues. The concentrations of IQP-0528 in each of these sites are illustrated separately in Fig. 1.

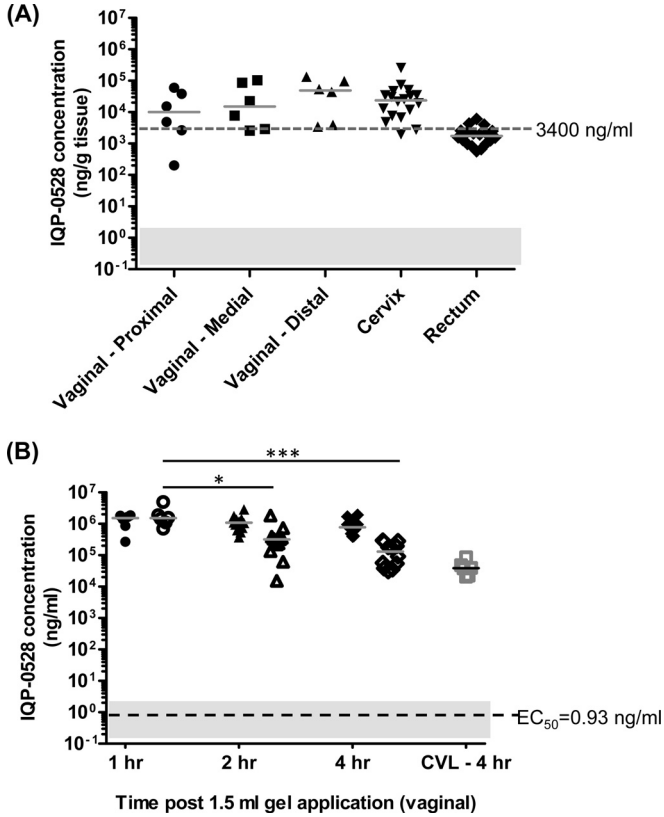

FIG 1 Concentrations of IQP-0528 in tissues at the time of necropsy (4 h) (A) and in vaginal fluids from spears/sponges collected from proximal (filled black symbols) and distal (open black symbols) sites relative to the cervix (B) over the 4-h period after application of 1.5 ml of IQB3002 (B). Also shown are the drug levels in CVL fluid (open gray symbols) at the time of necropsy. Medians and degrees of statistical significance ($P < 0.05$) are indicated by the gray bars and asterisks, respectively. The shaded region indicates the *in vitro* EC_{50} range of IQB3002 (against CCR5-tropic virus subtypes A to G) (22). The gray dotted line indicates the lowest tested concentration at which IQB3002 protected ectocervical and colorectal tissue explants *ex vivo*, with the black dotted line indicating the EC_{50} of IQB3002 in the presence of simulated vaginal fluid *in vitro* (22).

Cocultures of activated PBMCs with biopsy samples collected from proximal, medial, and distal sites relative to IQB3002 application were significantly protected from the HIV challenge (Fig. 2A) with viral inhibitions of 94, 89, and 90%, respectively. In contrast, coculture with biopsy tissue collected prior to IQB3002 application was either not protective or enhanced HIV p24 detection 10 days postchallenge. Importantly, we did not observe any decrease in the ability of previously frozen tissue to afford protection to cocultured target cells relative to that for cocultures using fresh biopsy specimens (Fig. 2B).

DISCUSSION

In this study, a novel IQB3002 gel product containing 1% IQP-0528 was evaluated for PK and PD in female RM using a 1.5-ml vaginal dose, which is a smaller volume than had been previously reported in HIV microbicide gel studies involving RM. There were undetectable levels of IQP-0528 in plasma, which is advantageous, as limiting the plasma drug circulation from topical microbicides will minimize systemic side effects and concerns about the development of drug resistance. Detailed analysis of fluid and tissue

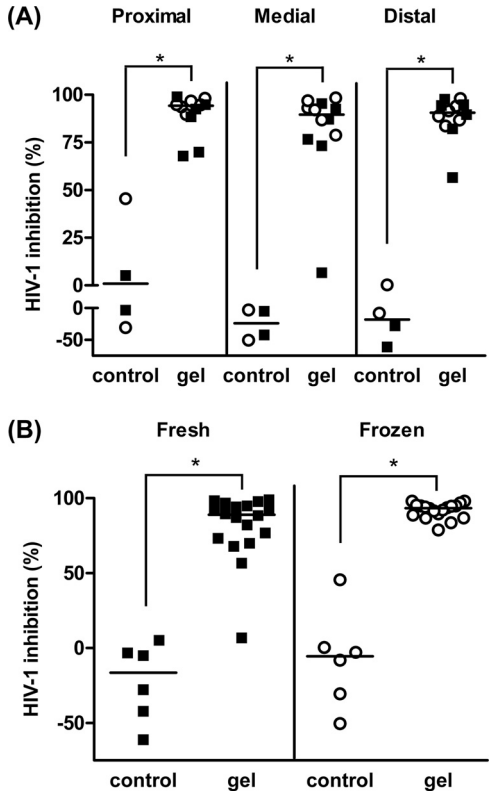

FIG 2 (A) Vaginal biopsy specimens (proximal, medial, and distal to the site of gel exposure) were collected at baseline ($n = 2$) and 4 h after gel application ($n = 6$) and were shipped to the Herold laboratory either frozen (○) or at 4°C in 200 μl of complete medium (■). Biopsy specimens were minced and cocultured for 3 days with phytohemagglutinin (PHA)-activated human PBMCs (1×10^5 cells/well) in triplicate wells of round-bottomed 96-well plates in the presence of culture medium used during shipment. Cocultures were challenged with HIV-1_{BaL} (approximately 10^3 $TCID_{50}$) for 2 h. The inoculum was removed, and cocultures were washed three times with serum-free medium and incubated for 10 days at 37°C. HIV replication was assessed by measuring p24 antigen in culture supernatants 10 days postinfection. (B) Comparison of HIV-1 inhibition in fresh and frozen biopsy specimens. Shown are the percentages of inhibition relative to those of PBMCs challenged in the absence of the biopsy coculture and are means obtained from one experiment where each supernatant was assessed in triplicate. *Statistically significant differences ($P < 0.01$, Mann-Whitney test). Bars indicate medians.

samples from the vaginal compartment showed that high concentrations of IQP-0528 were achieved over a 4-h time period. It is important to note that while the PK analysis indicates IQP-0528 concentrations in fluids and tissues, it is also a measure of gel dispersion and retention in the vaginal compartment. Indeed, traces of gel were observed on Weck-Cel spears and multiswab sponges at up to 4 h after gel application, with none being visibly evident at 24 h. The calculation of the half-life is not possible with our data set. IQP-0528 was not detected in plasma, and the drug concentrations detected in the vaginal compartment are a mix of what is present in vaginal fluid content and gel retained. Nonetheless, the IQP-0528 levels described above were observed in proximal, medial, and distal sites relative to the cervix, suggesting that the effective distribution of IQP-0528 can be achieved with a 1.5-ml volume of IQB3002 gel in RM. The median IQP-0528 concentrations observed in the vaginal compartment are up to 7 orders of magnitude higher than the *in vitro* EC_{50} (0.21 ng/ml to 1.29 ng/ml in human PBMCs challenged with CCR5-tropic virus subtypes A to G in the presence of IQB3002) (22). Similarly, these concentrations are also above the EC_{50} of IQB3002 in the presence of simulated vaginal fluid *in vitro* (22). Furthermore, the median drug levels in vaginal tissues exceed the lowest concentration (10 μM [3,400 ng/ml]) at which IQB3002 was previously shown to protect human ectocervical explant tissues from HIV-1 *ex vivo* (22) and are at levels that would be potentially inhibitory to HIV-1 infection. Indeed, the *ex vivo* PD assays described herein demonstrated this. It is unclear whether a smaller volume of gel would be as effective for drug delivery in the vaginal canal as a larger bolus. There may be a volume threshold that, once met, offers a sufficient degree of gel spread, retention, and/or absorption in the vaginal tract, and 1.5 ml may be enough to achieve this in RM. A previous study using computational modeling showed that a 4-ml volume of IQB3002 gel was capable of coating 84 cm^2 of vaginal surface area after 2 h of spreading (22). The study further showed that IQB3002, evaluated using the Franz cell apparatus and MatTek EpiVaginal tissue, had a permeation rate of 22.85 ± 0.19 μg/cm^3 h and displayed permeation of drug per volume of tissue at 65.89 ± 8.33 μg/cm^3 (22). However, gel volume is just one of many factors influencing gel deployment in the vaginal canal (42), and further characterization of the vaginal wall and gel properties in tandem is required to fully understand the fluid mechanics of gel movement and drug dosing in this biological compartment. Further titration of microbicide gel volumes is also warranted, and it would be interesting to pursue the feasibility of using smaller volumes in future microbicide studies to determine if favorable PK/PD profiles can be achieved with gels formulated with other API(s). Of note, we have previously shown that up to 4 h after a 1-ml vaginal dose of 1% TFV gel in pigtailed macaques, TFV levels in vaginal tissues were higher than those reported to be protective in the CAPRISA 004 trial (43, 44).

Consistent with the findings with tenofovir gels in RM (32), bidirectional dosing was evident by the detection of IQP-0528 in rectal tissues after vaginal application of 1.5 ml of IQB3002 gel, but concentrations were up to 1 log lower than those in vaginal tissues. The detection of IQP-0528 in rectal tissue is likely the result of drug diffusion and/or transport across compartments and not gel dispersion or leakage from the vagina to the rectum, particularly given the small volume that was applied vaginally and the lack of proximity between the gel application site and rectal fluid/tissue sampling. Bidirectional dosing is a desirable feature of microbicide gels, particularly if women apply the product in only the vaginal compartment but also engage in unprotected RAI during the same sexual event. However, it will be important to determine in efficacy studies if the lower drug exposure achieved in rectal tissue after vaginal dosing is protective against rectal virus challenge *in vivo*. Rectal efficacy by vaginal gel dosing may be hard to achieve given the lower drug exposure and the large mucosal surface area to protect in the rectum. However, the median IQP-0528 concentration observed in the rectal tissues after vaginal dosing with 1.5 ml of IQB3002 gel is up to 4 orders of magnitude higher than the *in vitro* EC_{50} (0.21 ng/ml to 1.29 ng/ml) (22), albeit below the lowest concentration (3,400 ng/ml) at which IQB3002 was previously shown to protect human colorectal explant tissues from HIV-1 *ex vivo* (22). Alternatively, women may choose to apply IQB3002 gel directly in the rectum since its formulation is balanced for application in both compartments (22). Additional rectal dosing studies are warranted to evaluate the optimal rectal

gel volume, as well as the PK and PD of the IQB3002 in this compartment in RM.

Consistent with our previous work with an intravaginal ring formulation of IQP-0528, biopsy specimens collected postdosing retained IQP-0528, which was subsequently released and internalized by cocultured PBMCs, providing significant protection from HIV challenge (27).

We previously assessed the effect of freezing biopsy specimens exposed to IQP-0528 *ex vivo* prior to coculture, and the data presented here validate those findings (27). This is the first instance where the effect of freezing samples from an *in vivo* study was evaluated. The fact that there is no loss of activity following frozen storage reflects drug stability and warrants the use of the coculture assay for the evaluation of tissue PD in samples from clinical trials conducted in sites where shipment to a central laboratory is required. A limitation of the coculture assay is that it relies on the ability of drugs to transit between exposed tissue and target cells. Also for this reason, tissues were not washed before use in the coculture assay, as this would wash out IQP-0528, which is not retained intracellularly and is rapidly transported in and out of cells. The results therefore do not distinguish between the antiviral activity of the tissue drug levels and gel retained on tissues but nonetheless collectively indicate the inhibitory effects achievable by the presence of drug in the vaginal compartment. Furthermore, because of the slow release kinetics, the PD of drugs that require intracellular phosphorylation such as nucleoside reverse transcriptase inhibitors should be evaluated using alternative methods (direct tissue challenge or antiviral activity of luminal fluid), Nevertheless, as shown here, the coculture assay may be very useful to assess NNRTI PD in samples collected from future animal studies and human clinical trials.

In summary, the data presented herein suggest that 1.5 ml of IQB3002 gel delivers IQP-0528 throughout the RM vaginal compartment at levels that are highly inhibitory to HIV-1 infection, as shown by the *ex vivo* coculture assay. High levels of drug were also observed in rectal tissues using this gel volume, suggesting that drug absorption after vaginal application may provide some level of protection from rectal exposures. The favorable results obtained with vaginal application of IQB3002 gel in RM support further exploration of the PK and PD profiles of different gel volumes in the vaginal compartment and evaluation of IQB3002 PK and PD following rectal application. The volume of gel to be used rectally to achieve the best balance of drug delivery and retention is still unknown, and future studies will be also be designed to fully investigate the half-life of IQP-0528 in the IQB3002 formulation. IQP0528 only partially inhibits SIV reverse transcriptase (RT) but is very effective against HIV-1 reverse transcriptase. Preclinical efficacy studies involving virus challenge of macaques with RT-SHIV162P3, the dual SHIV containing both HIV RT and a CCR5-specific HIV envelope gene in a simian immunodeficiency virus backbone (45, 46), are also warranted and would inform the field not only about product efficacy but also about whether any useful correlations can be drawn between *in vivo* efficacy studies and the *ex vivo* tissue coculture pharmacodynamic assay demonstrated herein.

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