Evaluation of Candidate Topical Microbicides in Pharmacokinetic and Pharmacodynamic *In Vitro* Models to Predict the Necessary Concentration Required to Prevent HIV Infection

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ABSTRACT

The identification and subsequent development of a successful microbicide product to prevent the transmission of sexually transmitted organisms is dependent on the robustness of the efficacy and safety algorithms that are used to advance products. Preclinical and clinical experiences have driven the natural evolution of these algorithms over time and it is understood that these will continue to change in order to adapt to the evolving field. It is well accepted that for a microbicide to be successful it must be at the right place, at the right time, and at the right concentration. *In vitro* pharmacokinetic models have been developed to assess the concentration of a microbicide that is able to permeate through representative cell monolayers to the tissues where the microbicide must accumulate in order to be effective. We have utilized these *in vitro* models, as well as *ex vivo* models including ectocervical and colorectal explants as a means to quantify the required concentration of a microbicide to prevent the transmission of HIV in target cells and tissues. Our data suggest that the candidate microbicide IQP-0528 rapidly penetrates through epithelial cells and explant tissues and achieves an inhibitory concentration. Conversely, the approved antiretroviral Tenofovir was found to be significantly slower in penetrating through epithelial cell monolayers and accumulating at the site of infection. Our data serves to correlate the "sterilizing" concentration of products as determined in the microbicide transmission and sterilization assay (MTSA) to that of the microbicides that have been shown to penetrate cell monolayers *in vitro,* plus those concentrations necessary to protect cervical and rectal explants from infection for these two compounds and other candidate microbicides representing different classes of anti-HIV inhibitors. We believe these assays will better predict the required protective concentration of a microbicide in target cells and tissues to prevent infection and will better inform animal modeling and human clinical trial dosing regimens.

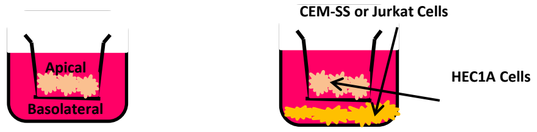
METHODOLOGY

Microbicide Transmission and Sterilization Assay (MTSA)

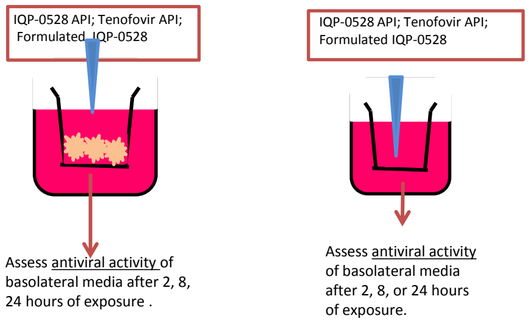
CEM-SS cells were infected with HIV-1_{IIIB} and incubated with 6 concentrations of compound. Every three days the cells were passaged by adding 1 mL of the infected culture with 4 mL of fresh CEM-SS cells while maintaining a fixed concentration of test compound. At each passage microscopic observations of syncytium formation and quantitation of cell-free virus by RT assay was monitored. Following 10 passages in the presence of compound, the cells were cultured in the same fashion without the addition of compound.

HEC1A Cytotoxicity Evaluation: HEC1A cells were seeded at a density of 1.0 x 10^5 cells/well in a 96-well plate in addition to a 24-well plate insert (0.4 µm-pore polyester) to monitor monolayer integrity. Cells were monitored for their trans-epithelial resistance (TEER) daily using a Millicell-ERS voltohmmeter (Millipore) until they reached TEER plateau (4-5 days). TEER plateau is designated as ~350 Ω•cm^2 after background media correction indicating the presence of an intact and impervious cell monolayer. The HEC1A monolayer in the 96-well dish was then exposed to IQP-0528 API, Tenofovir, formulated IQP-0528 or cellular media alone for 18 hours, after which the cells were washed 3X with cellular media, then replaced with fresh cellular media. The cells were allowed to incubate for 6 days (media changed every 3 days) at 37˚C at which time they were stained with XTT for cytotoxicity evaluation.

IQP-0528 API, Tenofovir API, or Formulated IQP-0528 Permeation--Cell Protection from HIV Infection: HEC1A cells were seeded at a density of 1.0 x 10^5 cells/well on the apical chamber of a 24-well plate insert/transwell and allowed to reach TEER plateau (~350 Ω•cm^2). Upon reaching TEER plateau HEC1A were exposed to IQP-0528 API, Tenofovir API, formulated IQP-0528 or cellular media alone for 2, 8, and 24 hours. At the time of API or microbicide addition to the apical chamber, Jurkat or CEM-SS cells were added at a density of 2.5 x 10^4 cells/well into the basolateral chamber. Following exposure at 2,8, or 24 hours, the compound was removed, followed by infection of cells in the basolateral chamber with a predetermined titer of the CXCR4 tropic HIV-1_{IIIB} or CCR5 tropic HIV-1_{Ba-L}. The infection was monitored and viral supernatant samples were collected daily up to 7-8 days. Viral replication was evaluated by measuring reverse transcriptase in the cellular supernatant. The experiment was also performed without HEC1A cells (- HEC1A) to assess the direct effects of application on protection of cells from HIV infection.



IQP-0528 API, Tenofovir API, or Formulated 1% IQP-0528 Gel Permeation-Antiviral Activity: HEC1A cells were seeded and monitored as stated above and allowed to reach TEER plateau. Cells were exposed to IQP-0528 API, Tenofovir API or formulated 1% IQP-0528 for 2, 4, 8, and 24 hours after which the basolateral chamber media was collected and assessed for antiviral properties through the following antiviral assays:



HIV Cytoprotection Assay: Following a six day acute infection of CEM-SS cells with the CXCR4-tropic IIIB strain of HIV-1 in the presence of compound, cell viability was measured spectrophotometrically (450/650 nm absorbance) using XTT dye reduction.

Acknowledgements

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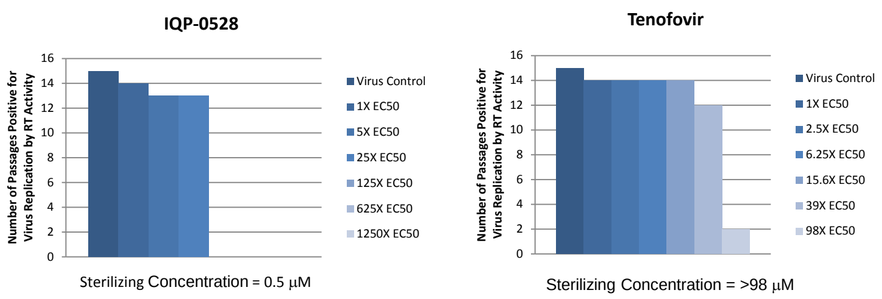
RESULTS

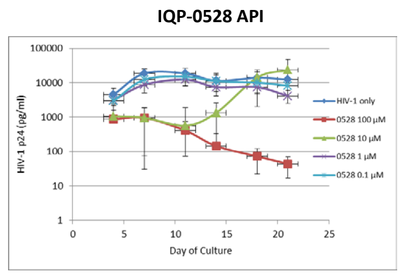
Anti-HIV Activity of Candidate Microbicides

Antiviral Evaluation	IQP-0528 EC_{50} (µM)	Tenofovir EC_{50} (µM)
CEM-SS/HIV-1_{IIIB}	0.004	1.0
PBMC/HIV-$1_{97/ZA/003}$	0.005	0.66

Sterilizing Concentration of Candidate Microbicides

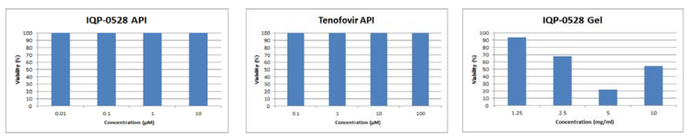


Inhibitory Concentration of a Candidate Microbicide in *Ex Vivo* Cervical Explants



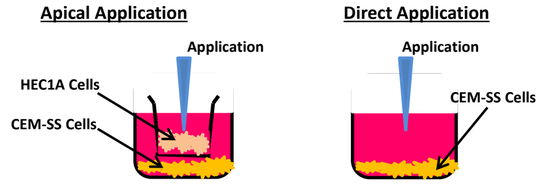
The MTSA-derived sterilizing concentration was 0.5 µM (above), which is the concentration required to completely suppress the transmission of virus in cell culture. The explant data (left) shows that a concentration of 100 µM in the unformulated API was was required to suppress virus in the *ex vivo* assay, indicating that a dose of 100 µM of API is needed in order to deliver the sterilizing concentration to the target tissue. Other data suggests better permeation of tissue by formulated API. Thus, delivery of formulated API may require doses 10-100-fold greater than the sterilizing concentration defined in the MTSA) and the MTSA may provide valuable and critical information on the dose concentration of API required for an effective microbicide. These data would suggest, as observed in clinical trials, complete protection/sterilization with Tenofovir will be difficult to achieve, potentially requiring a dose of >980 µM.

Cytotoxicity of API and Gel to HEC1A Cells



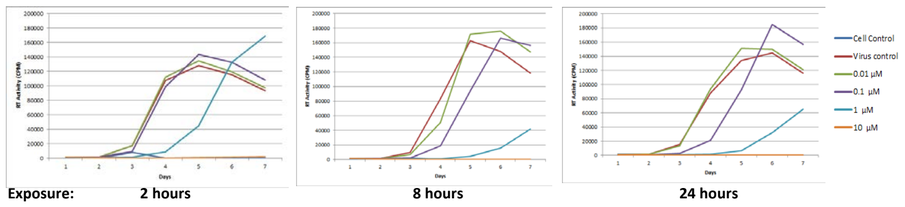
Permeation: Apical or Direct Application--Cell Protection from HIV Infection

Apical Application **Direct Application**



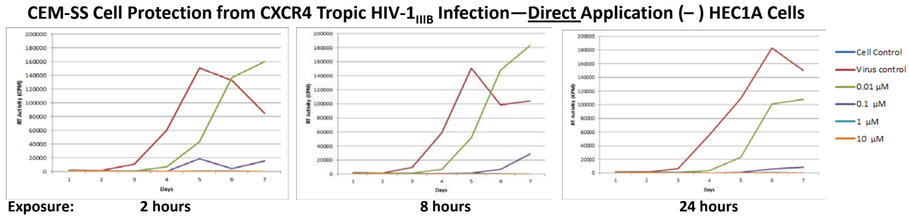
Permeation—Cell Protection from HIV Infection – IQP-0528 API

CEM-SS Cell Protection from CXCR4 Tropic HIV-1_{IIIB} Infection—Apical Application (+) HEC1A Cells



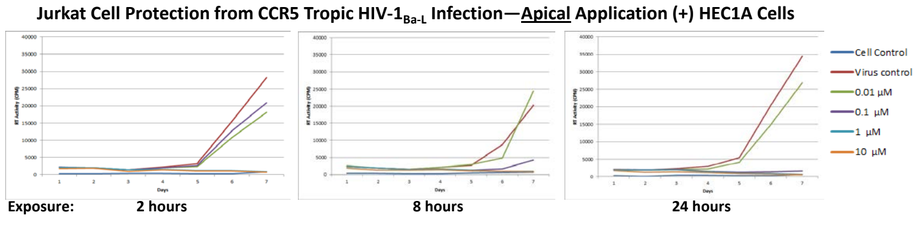
Exposure: 2 hours 8 hours 24 hours

CEM-SS Cell Protection from CXCR4 Tropic HIV-1_{IIIB} Infection—Direct Application (–) HEC1A Cells



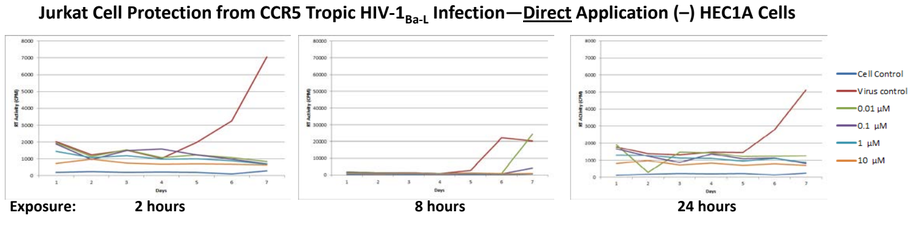
Exposure: 2 hours 8 hours 24 hours

Jurkat Cell Protection from CCR5 Tropic HIV-1_{Ba-L} Infection—Apical Application (+) HEC1A Cells



Exposure: 2 hours 8 hours 24 hours

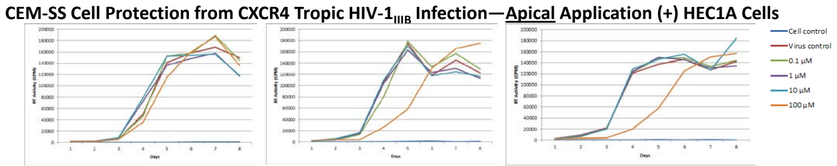
Jurkat Cell Protection from CCR5 Tropic HIV-1_{Ba-L} Infection—Direct Application (–) HEC1A Cells



Exposure: 2 hours 8 hours 24 hours

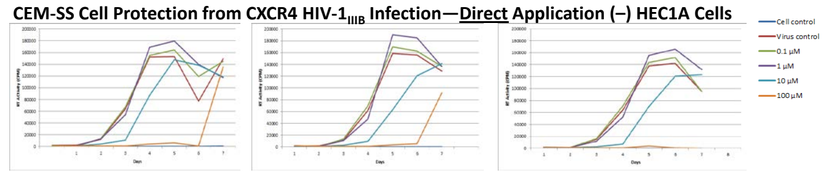
Permeation—Cell Protection from HIV Infection – Tenofovir API

CEM-SS Cell Protection from CXCR4 Tropic HIV-1_{IIIB} Infection—Apical Application (+) HEC1A Cells



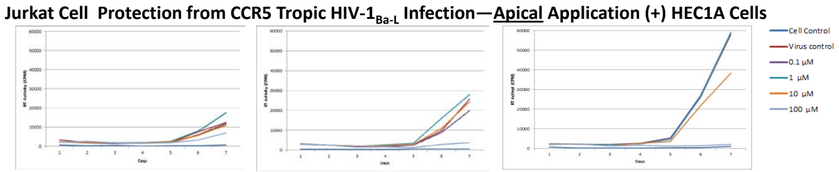
Exposure: 2 hours 8 hours 24 hours

CEM-SS Cell Protection from CXCR4 Tropic HIV-1_{IIIB} Infection—Direct Application (–) HEC1A Cells



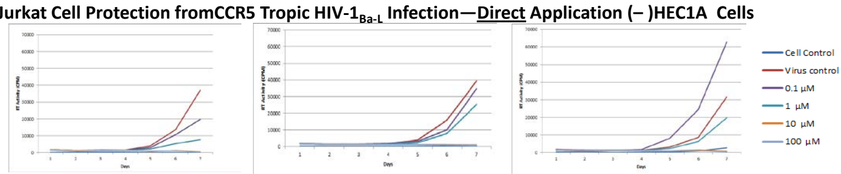
Exposure: 2 hours 8 hours 24 hours

Jurkat Cell Protection from CCR5 Tropic HIV-1_{Ba-L} Infection—Apical Application (+) HEC1A Cells



Exposure: 2 hours 8 hours 24 hours

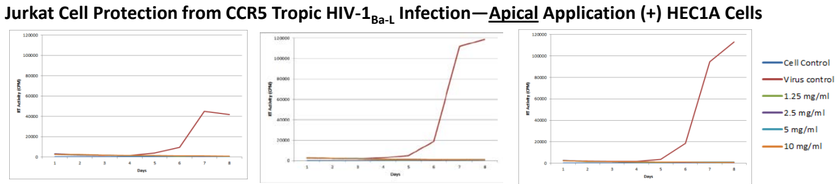
Jurkat Cell Protection fromCCR5 Tropic HIV-1_{Ba-L} Infection—Direct Application (–)HEC1A Cells



Exposure: 2 hours 8 hours 24 hours

Permeation Of Formulated IQP-0528 from 1% HEC Gel

Jurkat Cell Protection from CCR5 Tropic HIV-1_{Ba-L} Infection—Apical Application (+) HEC1A Cells



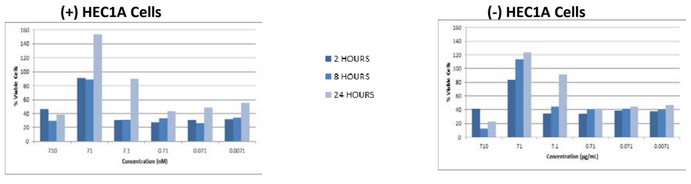
Exposure: 2 hours 8 hours 24 hours

Pharmacokinetics/Pharmacodynamics of IQP-0528 or Tenofovir

IQP-0528 API or Tenofovir API Application (+ HEC1A Cells)
Antiviral Bioassay of Basolateral Medium Against CXCR4-Tropic HIV-1_{IIIB} in CEM-SS cells

IQP-0528 API or Tenofovir API Application (-HEC1A Cells)
Antiviral Bioassay of Basolateral Medium against CXCR4-Tropic HIV-1_{IIIB} in CEM-SS cells

Formulated 1% IQP-0528 Gel Application (+HEC1A Cells)
Antiviral Bioassay of Basolateral Medium against CXCR4-Tropic HIV-1_{IIIB} in CEM-SS cells



Conclusions

- Explant and MTSA data suggest that microbicide dosing will require the delivery of sufficiently high concentrations of API in order to achieve a cell and tissue based sterilizing concentration of API. With IQP-0528, delivery of a formulated 100 µg/mL gel (1% gel) will provide a tissue concentration near the 0.5 µM sterilizing concentration.
- IQP-0528 API and Tenofovir API are non-toxic to HEC1A cells at all evaluated concentrations. 1% IQP-0528 gel is toxic to HEC1A cells at concentrations of 1 and 0.1%.
- IQP-0528 API effectively permeates through HEC1A cell monolayers at a concentration of 10 µM independent of exposure time thereby protecting target cells (CEM-SS and Jurkat) from infection in the basolateral chamber. IQP-0528 at 1 µM yields time dependent protection from infection, becoming effective after 8 hrs of exposure (CEM-SS cells).
- Tenofovir effectively permeates through HEC1A cell monolayers at a concentration of 100 µM independent of exposure time thereby protecting target cells (CEM-SS) from infection in the basolateral chamber; Tenofovir at 10 µM yields time-dependent protection (>8 hrs exposure time). In Jurkat cells infected with CCR5-tropic HIV-1_{Ba-L}, there is a delay in protection of cells at the same concentrations.
- 1% IQP-0528 gel is effective at preventing infection of Jurkat cells at non-toxic concentrations.
- Direct antiviral activity of IQP-0528 API and Tenofovir API is dependent on the presence of a HEC1A monolayer in addition to time. The same is true for 1% IQP-0528 gel.