Transport of Drug and Virus in the Intravaginal Ring Treated Pigtail Macaque FRT

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BACKGROUND

Prevention of HIV transmission using a tenofovir disoproxil fumarate (TDF)-eluting intravaginal ring (IVR) requires the right drug at the right place at the right time. For the first time, we demonstrate the ability to locate the initial site of infection and measure the drug distribution within an IVR-protected macaque FRT. This work demonstrates that not only is virus capable of infecting the ovaries, but that an IVR is also sometimes capable of achieving low drug concentrations there as well, highlighting the complexity of transport within the FRT and the need for further experiments and modeling.

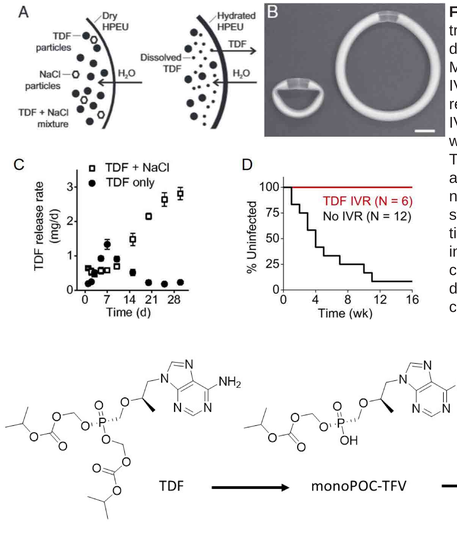

Figure 1. **A**. Schematic of intravaginal ring for sustained delivery of TDF for PrEP. **B**. Macaque and human TDF-IVR's used in trials. **C**. *In vitro* release rate of TDF from the IVR with TDF only and TDF with osmotic attractant. **D**. The TDF IVR protects macaques against repeated weekly vaginal challenge with SHIV162p3 starting 6 days after IVR insertion. Control macaques were infected after a median of four challenges. No infection was detected in IVR-protected macaques.

Figure 2. Structures of Tenofovir Disoproxil Fumarate, the prodrug of tenofovir; monoPOC-TFV, the intermediate metabolite of TDF; and tenofovir. TDF enters the cell and is hydrolyzed twice to form TFV.

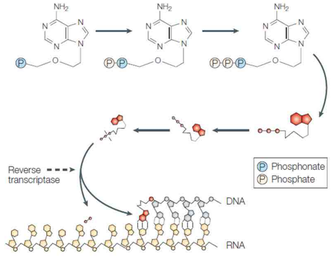

Figure 3. Protection against HIV with tenofovir is accomplished when TFV is converted to tenofovir diphosphate inside cells. TFV-DP competes with its natural nucleotide complement deoxyadenosine 5'-triphosphate for incorporation into a growing HIV DNA chain. Upon incorporation, the HIV DNA chain elongation terminates.

METHODS

Three pigtail macaques were treated with TDF-IVRs for 28 days, and vaginally challenged with a high dose of a replicative SIVmac239 virus and a single round non-replicative SIV-based vector expressing Luciferase and mCherry reporter genes (JRFL pseudotyped LICh vector). The isolated FRT was treated with luciferin to detect Luciferase activity using IVIS, allowing us to identify the initial site of the infection. The tissue was sectioned and imaged further under IVIS. Tissue samples were processed and TFV and TFV-DP concentrations were quantified using LC-MS/MS, with ^{13}C-labeled TFV as an internal standard.

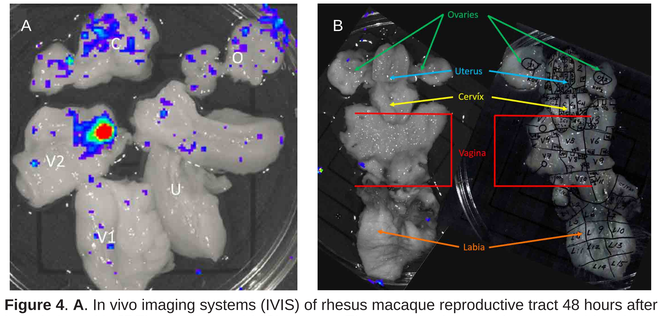

Figure 4. **A**. In vivo imaging systems (IVIS) of rhesus macaque reproductive tract 48 hours after inoculation with JRFL pseudotyped LICh vector. The reproductive tract is separated into large sections (panel 1; V1: labia and lower vagina; V2: upper vagina; C: cervix; U: uterus; O: ovary), soaked in d-luciferin to identify luciferase expressing regions with IVIS, and dissected into sequentially smaller pieces (panels 2–4) to isolate strongly luminescent regions measuring 2×2 mm^2. **B**. Parts of the macaque female reproductive tract (FRT).

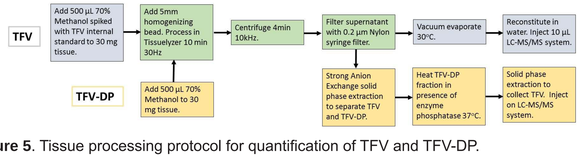

Figure 5. Tissue processing protocol for quantification of TFV and TFV-DP.

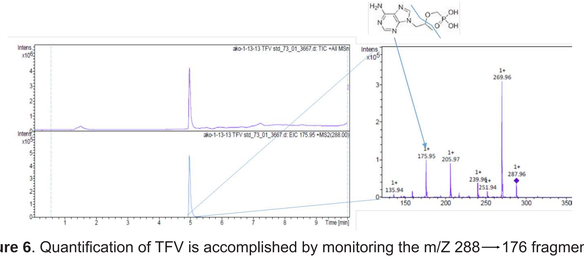

Figure 6. Quantification of TFV is accomplished by monitoring the m/Z 288⟶176 fragment with HPLC-MS/MS in positive ion mode. The LLOQ for TFV is 63 fmol/mg tissue.

RESULTS

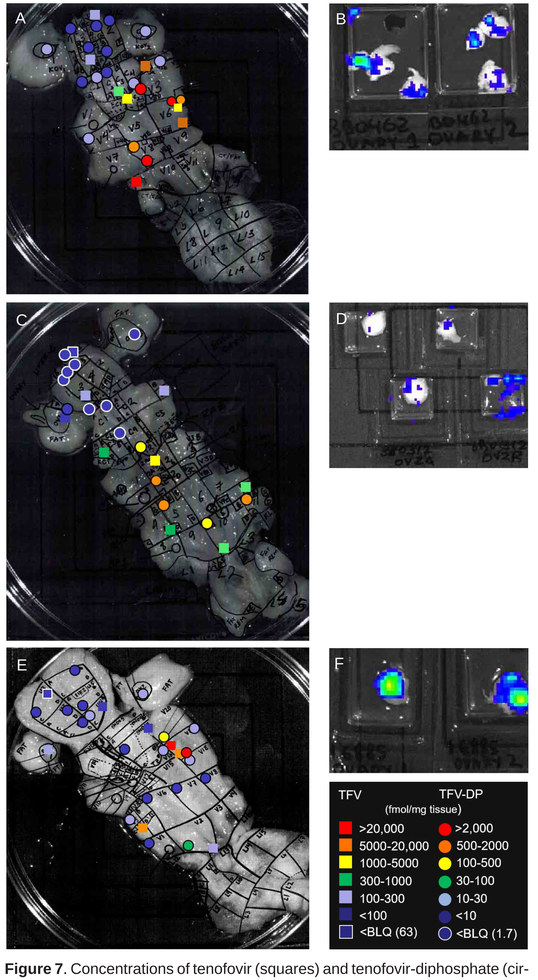

Figure 7. Concentrations of tenofovir (squares) and tenofovir-diphosphate (circles) in FRT of animal BB0462 (**A**), 1.6885 (**C**) and BB0312 (**E**). IVIS imaging of the ovaries of animal BB0462 (**B**), 1.6885 (**D**) and BB0312 (**F**).

Infection events were detected in both ovaries in two animals and in one ovary of the third macaque using IVIS. TFV levels were found to be variable throughout the FRT, with the highest concentrations found in the upper vagina/lower cervical area, near the site of the ring. The concentration of TFV in the FRT of the macaques was in the range of 40-28,000 fmol/mg of tissue and the concentration of TFV-DP in the FRT of the macaques was in the range of 3-6000 fmol/mg of tissue. In the uterus and ovaries, the TFV concentration is at or near the IC_{50} of TFV in PBMC's.

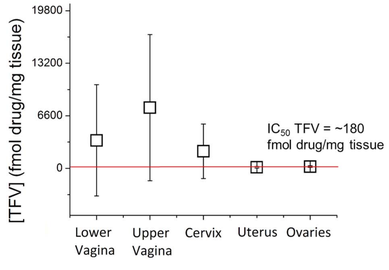

Figure 8. Average TFV levels in the FRTs of TDF-IVR treated pigtail macaques.

CONCLUSIONS

- Utilizing reporter viruses allows us to perform complete pharmacokinetic and pharmacodynamic studies at both the anatomical and cellular levels.
- Our results demonstrate the gradient of tissue drug levels emanating from the site of the ring. We observe lower drug levels in the upper reproductive tract for all animals.
- Infection events were seen in the ovaries of all three macaques, possibly reflecting the variability of ovarian drug levels (range of 7-30 fmol TFV-DP/mg of tissue) and virus transport.

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FURTHER INFORMATION

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