Drug concentrations after topical and oral antiretroviral pre-exposure prophylaxis: implications for HIV prevention in women

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The early closure of a clinical trial assessing the effectiveness of oral antiretroviral pre-exposure prophylaxis (PrEP) in women, FEM-PrEP,[1] is a substantial setback for HIV prevention. Expectations of this trial were high in view of favourable results from the pre-exposure prophylaxis initiative (iPrEX) trial,[2] which studied the same drug and dosing strategy in men who have sex with men, and the Centre for the AIDS Programme of Research in South Africa (CAPRISA 004) trial,[3] which tested tenofovir gel (a topical PrEP formulation) in heterosexual women. As a result, the interim FEM-PrEP trial results, announced on April 18, 2011, which showed no protection against HIV infection,[1] were disappointing. Using publicly available information[1] and data from other PrEP studies, we offer a potential explanation for the results of the FEM-PrEP trial.

In high HIV prevalence settings, such as sub-Saharan Africa, young women have disproportionately high HIV incidence rates, up to 8-times higher than for men of the same age.[4] Available HIV prevention strategies provide few options for young women who are at high risk of infection but who are unable to convince their partner to be faithful or use condoms, underscoring the urgent need for a women-initiated HIV prevention technology. To this end, several microbicide trials have been undertaken during the past 17 years. Until 2010, none had shown protection against HIV acquisition.[5] A new approach was needed. Antiretroviral drugs, already shown to be effective in treating HIV infection and prevention of mother-to-child transmission, heralded a new option to prevent sexual transmission.

FEM-PrEP was a phase 3, double-blind, randomised, placebo-controlled trial assessing the effectiveness of daily oral tenofovir disoproxil fumarate and emtricitabine for prevention of HIV acquisition in women aged 18–35 years in South Africa, Kenya, and Tanzania. At a scheduled interim analysis, the HIV incidence rate was 5 per 100 person-years in the 1951 women enrolled, and the 56 HIV endpoints were equally distributed between the study groups.[1] Continuation of the study to the planned 72 HIV endpoints in an attempt to show effectiveness was deemed futile, so the decision was made to undertake an orderly closure of the trial.

Why did the FEM-PrEP trial not show protection against HIV infection? To conclude that oral tenofovir disoproxil fumarate and emtricitabine does not prevent HIV infection in women would be overly simplistic and premature; several possible explanations exist for the reported primary HIV outcome in the trial. Such results could have occurred by chance in a trial of a truly effective product, but the chance of observing no effectiveness if the drug is truly 50% protective against HIV is about 3 in 1000. However, two of the most plausible explanations for the trial results are low pill adherence and inadequate drug concentrations at the site of infection—ie, the genital tract.

Adherence and drug distribution are only two of the many components of the pathway that is intended to end with antiretroviral drugs preventing the development of HIV infection after viral exposure in the female genital tract. However, they are both crucial for the desired outcome. Adherence levels are dependent on human behaviour in the context of the user's social environment, whereas the available concentration of the drug is affected by its pharmacological properties and host-cell biology.

Although reported adherence in the FEM-PrEP trial was high,[1] its accuracy cannot be assessed at this time. Blood concentrations of the drugs in women assigned to tenofovir disoproxil fumarate and emtricitabine and analysis of effectiveness, stratified by adherence levels, will provide a more reliable indication of pill adherence during the trial. A comparison of drug concentrations in the women who developed HIV infection with those in appropriately selected controls who remained uninfected during the course of the trial could likewise provide useful clues. In the meantime, exploration of alternate explanations is important, in the event that low adherence does not fully account for the trial's outcome.

Tenofovir and emtricitabine concentrations in genital and rectal tissues have been assessed in previous phase 1 and pharmacokinetic studies of the oral preparation and tenofovir gel. With orally administered drug, the median cervicovaginal fluid concentration of tenofovir and emtricitabine at the end of the 24 h dosing interval was 68 ng/mL (IQR 28–112) and 596 ng/mL (537–644), respectively.[6] Vaginal tissue concentrations of tenofovir and emtricitabine were 7 ng/g and 63 ng/g (ng/g is roughly equivalent to ng/mL), respectively.[7]

These concentrations from oral dosing are substantially lower than vaginal concentrations achieved with topical tenofovir gel or rectal concentrations from oral tenofovir disoproxil fumarate and emtricitabine. The median cervicovaginal fluid concentration of tenofovir at the end of the 24 h gel dosing interval was 100 000 ng/mL and the vaginal tissue concentration was 7000 ng/g.[8] Rectal tissue concentrations of tenofovir and emtricitabine 24 h

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	Vaginally administered 1% tenofovir gel	Orally administered tenofovir disoproxil fumarate
Blood		
Plasma[7,8,10,11]	~1–10 ng/mL	25–50 ng/mL
Peripheral blood mononuclear cell[11]	<10 fmol/10^6 cells	~70 fmol/10^6 cells
Mucosal fluid		
Cervicovaginal[7,8,10]	~10^5–10^6 ng/mL	~70 ng/mL
Tissue		
Vaginal (tenofovir)[7,8,10,11]	~10^3–10^4 ng/g	10 ng/g
Vaginal (tenofovir diphosphate)[7,8,10,11]	~10^3 fmol/mg	1–10 fmol/mg
Cytobrush cells (tenofovir diphosphate)[8,11]	~10^4–10^5 fmol/10^6 cells	~10^3 fmol/10^6 cells
Rectal (tenofovir)[7,11]	NA	2000 ng/g
Rectal (tenofovir diphosphate)[7,11]	NA	100–1000 fmol/mg

PBMC=peripheral blood mononuclear cell. NA=not available.

Table: **Tenofovir concentrations measured in blood, mucosal fluid, and tissue 24 h after topical or oral administration**

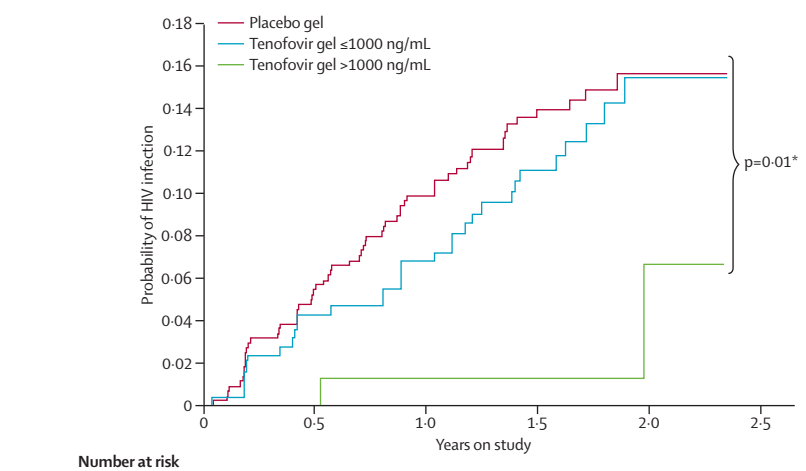

Figure: **HIV infection rates in women in the CAPRISA 004 trial[3]**
Women assigned to tenofovir gel are stratified by cervicovaginal fluid concentrations of tenofovir.
*Log rank comparing women with tenofovir concentrations greater than 1000 ng/mL versus placebo.

after a single dose of the oral drug were 1877 ng/g and 124 ng/g, respectively.

Since tenofovir diphosphate and emtricitabine triphosphate are the metabolites of the parent drugs that inhibit viral replication, their intracellular tissue concentrations after oral and topical dosing could be informative. Although threshold concentrations for protection against HIV infection have not yet been established, data from an ex-vivo colorectal biopsy infection model[9] suggest that at least 1000 fmol/mg of tenofovir diphosphate could be needed for near complete protection. The concentrations of this metabolite 24 h after one oral dose of tenofovir disoproxil fumarate and emtricitabine were 206 fmol/mg in rectal tissue and only about 2 fmol/mg in vaginal tissue.[7] By comparison, the tenofovir diphosphate concentrations 24 h after one tenofovir gel dose were about 1000 fmol/mg in vaginal tissue and roughly 10 000 fmol/10^6 cervical cells obtained from cytobrush sampling.[8] In short, tenofovir diphosphate concentrations are about 100-fold higher in rectal than vaginal tissues with oral tenofovir disoproxil fumarate and emtricitabine, and about 1000-fold higher in vaginal tissues with tenofovir gel than with oral tenofovir disoproxil fumarate and emtricitabine (table).

To gain insight into the potential threshold tenofovir concentrations needed to prevent HIV infection in women, we assessed tenofovir concentrations in undiluted aspirated cervicovaginal fluid with a validated ultra performance liquid chromatograph-mass spectrometry method in women assigned to tenofovir gel in the CAPRISA 004 trial.[3] Samples were available from the first study visit post infection from 34 of the 38 HIV seroconverters and from a randomly selected study visit from 301 women assigned to tenofovir gel who remained uninfected during the trial.

With data from this trial,[3] HIV incidence in women with tenofovir concentrations of 1000 ng/mL or less (n=253) and those with tenofovir concentrations of 1000 ng/mL or more (82) was compared with that in the placebo gel group (figure). The HIV incidence rate in women with tenofovir concentrations of 1000 ng/mL or less was close to that in the placebo group (7·8 *vs* 9·1 per 100 women-years; incidence rate ratio [IRR]=0·86, 95% CI 0·54–1·35, p=0·51). However, the HIV incidence rate in women with tenofovir concentrations greater than 1000 ng/mL was significantly lower than that in the placebo group (2·4 *vs* 9·1 per 100 person-years; IRR=0·26, 95% CI 0·05–0·80, p=0·01). Adjustment for age, study site, duration of study participation at the point when tenofovir concentration was measured, sexual frequency, and condom use did not materially change this finding.

These tenofovir concentrations are proxy markers of drug exposure at the actual time of HIV exposure, and some residual systematic differences between the three groups of women could account for some of the variations in HIV risk, despite adjustment for potential confounders. Notwithstanding, our data suggest that cervicovaginal fluid concentrations of tenofovir greater than 1000 ng/mL were required to prevent HIV infection. This value is more than ten times the concentration seen with oral tenofovir disoproxil fumarate and emtricitabine.

What are the implications for HIV prevention research? Detailed analyses of the FEM-PrEP[1] data will undoubtedly enhance our understanding of how antiretrovirals prevent HIV infection in women. In the interim, our suggestions for continuing and proposed PrEP research are: first, the effectiveness trials that are underway (registered with ClincalTrials.gov, numbers NCT00705679, NCT00557245, NCT00448669, NCT00119106) for tenofovir disoproxil fumarate alone and in combination with emtricitabine are crucially needed to corroborate or refute the FEM-PrEP[1] trial results and to provide information about HIV effectiveness in diverse populations, various

formulations, and in different routes of transmission. Researchers need to factor the FEM-PrEP trial outcome into the information provided to participants. Furthermore the data-review plans, especially the rules for futility, might need to be revisited.

Second, investigators need to revise existing or develop new animal-challenge and tissue models for PrEP to be able to assess varying drug dosages. Specifically, further animal models with which to assess vaginal challenge after oral dosing are needed. Additonally, the models might need an infectious virus inoculum that is closer to physiological values. Third, until there is improved clarity about the threshold concentration of tenofovir that is likely to protect against HIV, the goal in future clinical trials of this drug should be to attain the highest tolerable drug concentrations in the vagina. Options such as combinations of oral and topical formulations could be worth investigation, especially in settings in which anal sex is common in women. Fourth, efforts to enhance adherence in PrEP trials are crucial. Finally, new PrEP formulations, such as intravaginal rings and injectables, will need to be carefully assessed to work out whether the drug concentrations achieved at the site of viral exposure are likely to be high enough to prevent HIV infection.

The FEM-PrEP trial is a sharp reminder of the uncertainty of the scientific endeavour. Success needs an iterative approach to identify the most appropriate drugs, drug concentrations, adherence support, formulations, and dosing regimen for each route of HIV transmission. The proof of principle that antiretroviral drugs can prevent sexual transmission of HIV has reinvigorated HIV prevention. It has created new hope that antiretroviral-based PrEP strategies, especially those that are women-initiated, could in combination with other prevention interventions, finally stem the tide of the HIV pandemic.

Contributors

QAK and SSAK as Co-Principal Investigators conceived and designed the CAPRISA 004 trial. ADMK analysed the tenofovir concentrations in the cervicovaginal fluid. LW undertook the statistical analysis. SSAK prepared the first draft of the report and all authors contributed important edits and revisions.

Conflicts of interest

We declare that we have no conflicts of interest.

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