

Shadows Looming Over Tenofovir Gel Application

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The efficacy of the antiretroviral drug tenofovir, a phosphonate nucleoside analogue HIV reverse transcriptase inhibitor (NRTI), for HIV prevention in situations of high exposure has been tested in a number of trials. While the oral pill, alone or in combination with emtricitabine (Truvada®), has proven to provide protection, studies testing tenofovir gel applications have yielded mixed results. Lack of protection was mostly attributed to low adherence, but off-target effects of the drug on the mucosa could have contributed. Moreover, during topical use of antiretrovirals, very high local drug concentrations are achieved, warranting a comprehensive assessment of the mucosal changes induced by each candidate microbicide to ensure safety.

To address this question, Dr. Florian Hladik from the Vaccine and Infectious Disease Division (VIDD), in a joint effort with other investigators from Fred Hutch as well as other institutions in US and in Canada, conducted a systems biology study within the clinical trial MTN-007 (Protocol Chair: Dr. Ian McGowan, University of Pittsburgh), in which 65 participants were randomized to receive a rectal gel formulation containing tenofovir 1%, nonoxynol-9 (N-9) 2% (a detergent included as a positive toxicity control), no gel or no treatment. Furthermore, as tenofovir gel is a candidate product for HIV prevention in women, its effect on vaginal keratinocytes obtained from tissues discarded following vaginal repair surgery was also evaluated. "Topical microbicides, for example vaginal gels containing an antiviral drug, are an entirely new HIV prevention strategy for which no precedence exists. So it is prudent to broadly assess their safety, in particular given the high drug concentrations that are sometimes achieved in the mucosa" explained Dr. Hladik. The clinical study was reported in PLOS One in 2013; the results of the systems biology study and follow-up experiments were published in the February edition of *eLIFE*.

The effect of tenofovir gel on global gene expression profile was determined in rectal biopsies as well as in primary vaginal keratinocyte cultures. In the rectal mucosa, one week of daily tenofovir applications suppressed the expression of 505 genes and induced 137 genes. The effect of N-9 was much more limited in that it suppressed only 56 and induced 60 genes. Among the genes highly activated by tenofovir gel, the investigators identified several encoding for secretory proteins, such as the chemokines CCL2, CCL19, CCL21, CCL23, CXCL9 and CXCL13; correspondingly, a number of lymphocyte markers were increased. On the other hand, tenofovir inhibited the transcription of many genes encoding for transcription factors, as well as genes regulating cell proliferation,

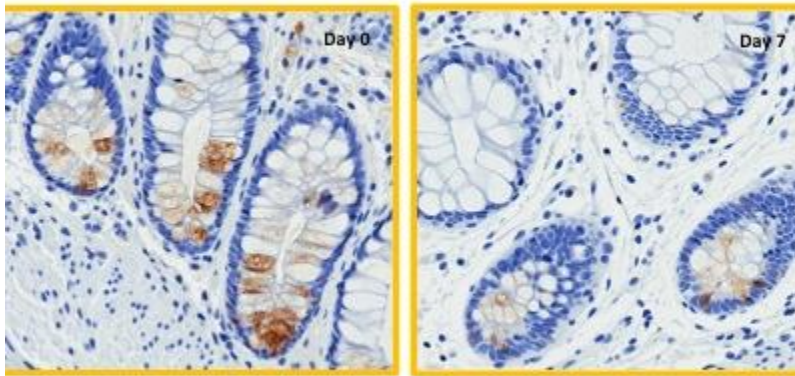
apoptosis and epithelial structure organization. Tenofovir also suppressed immunoregulatory molecules such as interleukin 10 (IL-10), a cytokine important for toning down inflammatory processes (see Figure). A similar pattern of gene expression modification was detected in cultures of vaginal keratinocytes treated with tenofovir in vitro. These cells also grew faster under the influence of tenofovir, indicating a hyperplastic effect of the drug.

Both in vivo and in vitro, tenofovir also suppressed the expression of PNPT1, a master regulator of RNA import into mitochondria. This observation led the investigators to explore the possibility that tenofovir affects mitochondrial functions. They observed a decrease in the transcription of mitochondrial DNA, and in two samples analyzed by electron microscopy found that the number of mitochondria significantly decreased. Mitochondrial toxicity of NRTIs is well known but as yet unexplained – the current data point to blockade of PNPT1 as a possible cause.

In conclusion, this study uncovered a number of potentially concerning effects of the antiretroviral drug tenofovir on the mucosa, although concrete clinical correlates of these activities are still lacking. Should tenofovir 1% gel become available as an HIV prevention tool, its safety over long-term use must be carefully monitored. The immunological, mitochondrial and hyperplastic properties of tenofovir at high concentrations could limit its usefulness as a topical microbicide and argue in favor of alternative drugs with a different mechanism of action.

"Our study shows that a broad systems biology approach can uncover potential safety concerns that need to be followed up upon. We also gained a lot of new information about the biology of the human vaginal and rectal mucosa, which will keep us busy for many years to come" concluded Dr. Hladik. His Lab is already planning follow-up studies, and we look forward to reporting future exciting results from this work.

[Hladik F, Burgener A, Ballweber L, Gottardo R, Vojtech L, Fourati S, Dai JY, Cameron MJ, Strobl J, Hughes SM, Hoesley C, Andrew P, Johnson S, Piper J, Friend DR, Ball TB, Cranston RD, Mayer KH, McElrath MJ, McGowan I.](#) 2015. Mucosal effects of tenofovir 1% gel. *Elife*, 4. doi: 10.7554/eLife.04525.



IL10

Image provided by Dr. Florian Hladik.

Immunohistochemistry visualization of IL10 production at day 0 and 7 of Tenofovir treatment in colon mucosa.