

PRO 2000 Gel Inhibits HIV and HSV Infection Following Vaginal Application: A Double Blind Placebo-Controlled Trial

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Abstract

Several candidate topical microbicides designed to prevent transmission of HIV and other STI are being advanced to large scale clinical trials based on activity observed *in vitro* and in animal infection models. However, there are as yet no data demonstrating retention of microbicide activity following human application. A prospective, randomized, double blind placebo-controlled study was conducted among 20 HIV-infected women to assess the anti-HIV and anti-HSV activity in cervicovaginal lavage (CVL) fluid collected 1 hour after a 2 g intravaginal dose of 0.5% PRO 2000 Gel, a leading candidate topical microbicide, or a matched Placebo Gel. CVL obtained after PRO 2000 Gel application significantly inhibited HIV infection of CCR5-expressing HeLa cells and primary human macrophages and HSV infection of human cervical cells compared to anti-viral activity in CVL obtained at screening, whereas the placebo gel had little or no anti-viral activity. HIV and HSV infection were reduced by > 1000-fold ($p < 0.001$ by unpaired two tailed t-test). The concentration of PRO 2000 in CVL post-application ranged from 114-341 µg/ml, a concentration that exceeds the IC_{50} of the drug. No increase in polymorphonuclear leukocytes, IL-1 β , IL-8 or SLP1 in CVL was observed. These results demonstrate that 0.5% PRO 2000 Gel is sufficiently bioavailable and retains substantial anti-viral activity one hour after intravaginal application. Moreover, this strategy provides a mechanism for testing *in vivo* the efficacy of a microbicide before embarking on large-scale clinical trials.

Background

- Women now make up nearly half of the 37 million adults, aged 15-49, with HIV worldwide.
- Unprotected sexual intercourse is the predominant mode of HIV transmission.
- Genital herpes plays a major role in the sexual spread of HIV.
- HSV-2 seroprevalence rates in developing countries range from 60-80% among young adults.
- Fully protective vaccines for HIV or HSV are unlikely to become available for years. Thus, there is an urgent need for novel preventative strategies.
- Topical microbicides, products designed for vaginal application to prevent STI, offer the potential to substantially reduce the rates of transmission of HIV & HSV.
- There is no data demonstrating that any microbicide retains anti-HIV or anti-HSV activity following intravaginal application.
- Little is known about subtle effects of compounds on the mucosal environment, including induction of inflammation and interference with host defense mechanisms.
- Microbicides may alter production of soluble factors that directly or indirectly protect against STI, such as secretory leukocyte protease inhibitor (SLPI).
- Laboratory assays to predict efficacy & safety of microbicides are needed.

Objectives

- To determine whether PRO 2000 Gel present in CVL obtained 1 h after vaginal application inhibits HIV and HSV infection using a spiking strategy
- Assess the acute inflammatory response to a single dose by measuring inflammatory cells, IL-1 β , IL-8 and SLP1 in CVL

Study Design

Participants: HIV-infected women between the ages of 18 and 50 years. Inclusion criteria: HIV-1 plasma RNA of $\sim 4.7 \log_{10}$ copies/ml by RT-PCR (Amplicor HIV-1 Monitor, version 1.5, Roche) within 2 months of screening, no change in antiretroviral therapy in the two months preceding participation, negative or ASCUS (atypical squamous cells of undetermined significance) Pap smear result within 6 months of participation. Exclusion criteria: pregnancy, breastfeeding, menopause, hormonal contraception or a recent history of intermenstrual bleeding, gynecological surgery, vaginitis, urinary tract infection, STI, or recent vaginal product use.

Protocol: 20 HIV-infected women were randomized in a double-blind manner to receive one intravaginal dose of 0.5% PRO 2000 gel or a matched placebo gel. CVL (10 cc normal saline) was performed at screening (48- h earlier) and one hour after application of drug or placebo. The anti-HIV and anti-HSV activity in CVL was determined using a spiking strategy and the concentration of PRO 2000 gel was determined by fluorescence.

Study drugs: PRO 2000 Gel is an aqueous vaginal gel formulation containing 0.5% (w/w) PRO 2000, a synthetic carbomer gelling agent (Carbopol[®]1382, Novon, Inc.), a lactic acid/trolammonium lactate buffer (pH 4.5) and preservatives. The human dose is approximately 2 mL containing 10 mg PRO 2000.

Results

Table 1. Subject demographics and clinical data

PRO 2000 GEL RECIPIENTS

Subject #	Age	Race/Ethnicity	CD4 (cells/mm3)	HIV-1 RNA (log10copies/ml)	Pap	CVL pH post-application	PRO 2000 µg/ml
2	44	Black	225	4.7	Negative	4.0	166
3	40	Black	366	4.6	Negative	5.0	151
5	36	Hispanic	407	4.7	Negative	4.0	342
6	43	Hispanic	*NS	4.9	Negative	4.0	262
9	39	Hispanic	122	5.1	ASCUS	4.0	184
12	42	Black	283	4.6	Negative	4.0	115
15	36	Black	353	5.0	Negative	4.5	185
16	47	Black	188	5.0	Negative	4.5	256
18	34	Asian	525	5.3	Negative	4.5	217
20	31	Black	375	4.2	Negative	4.5	173

PLACEBO GEL RECIPIENTS

Subject #	Age	Race/Ethnicity	CD4 (cells/mm3)	HIV-1 RNA (log10copies/ml)	Pap	CVL pH post-application
1	40	Hispanic	94	4.6	Negative	5.0
4	43	Hispanic	675	5.8	Negative	4.0
7	35	Black	42	5.1	ASCUS	4.0
8	40	Black	151	5.1	Negative	4.0
10	37	Hispanic	264	4.4	Negative	4.5
11	47	Hispanic	264	5.0	Negative	4.5
13	35	Hispanic	686	5.8	ASCUS	4.5
14	41	Black	8	5.3	Negative	4.5
17	31	Hispanic	185	5.1	ASCUS	4.5
19	29	Black	616	4.6	ASCUS	4.5

*QNS, quantity not sufficient. There were no statistically significant differences between subjects who received PRO 2000 Gel or Placebo Gel with respect to age, CD4 count, HIV viral load, or pH of CVL obtained post-gel application: mean age 40.2 vs. 37.8 years, mean CD4 count 305 vs. 300 cells/mm³, mean HIV-1 viral load 4.8 vs. 5.1 log₁₀copies/ml, mean CVL pH 4.3 vs. 4.4, respectively.

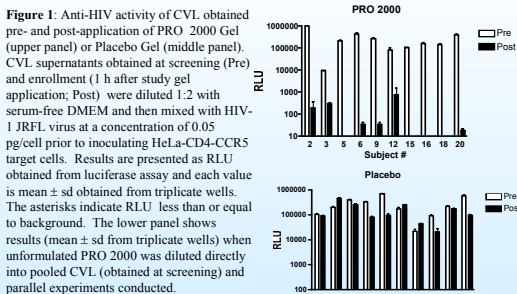


Figure 1: Anti-HIV activity of CVL obtained pre- and post-application of PRO 2000 Gel (upper panel) or Placebo Gel (middle panel). CVL supernatants obtained at screening (Pre) and enrollment (1 h after study gel application; Post) were diluted 1:2 with serum-free DMEM and then mixed with HIV-1 JRFL virus at a concentration of 0.05 pg/cell prior to inoculating HeLa-CD4-CCR5 target cells. Results are presented as RLU obtained from luciferase assay and each value is mean \pm sd obtained from triplicate wells. The asterisks indicate RLU less than or equal to background. The lower panel shows results (mean \pm sd from triplicate wells) when unformulated PRO 2000 was diluted directly into pooled CVL (obtained at screening) and parallel experiments conducted. The log fold reduction in HIV infection was 4.027 \pm 1.133 for the active gel group compared to 0.245 \pm 0.45 for the placebo group ($p < 0.001$, Wilcoxon test). The degree of anti-HIV activity observed in the CVL samples obtained post-application of PRO 2000 Gel is comparable to that observed with 100 µg/ml of unformulated PRO 2000 diluted in CVL.

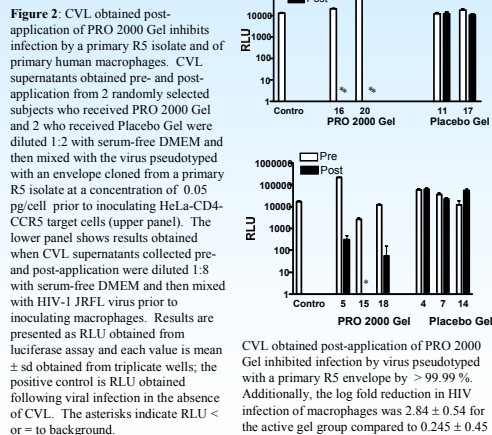


Figure 2: CVL obtained post-application of PRO 2000 Gel inhibits infection by a primary R5 isolate and by primary human macrophages. CVL supernatants obtained pre- and post-application from 2 randomly selected subjects who received PRO 2000 Gel and 2 who received Placebo Gel were diluted 1:2 with serum-free DMEM and then mixed with the virus pseudotyped with an envelope cloned from a primary R5 isolate at a concentration of 0.05 pg/cell prior to inoculating HeLa-CD4-CCR5 target cells (upper panel). The lower panel shows results obtained when CVL supernatants collected pre- and post-application were diluted 1:8 with serum-free DMEM and then mixed with HIV-1 JRFL virus prior to inoculating macrophages. Results are presented as RLU obtained from luciferase assay and each value is mean \pm sd obtained from triplicate wells; the positive control is RLU obtained following viral infection in the absence of CVL. The asterisks indicate RLU $<$ or = to background.

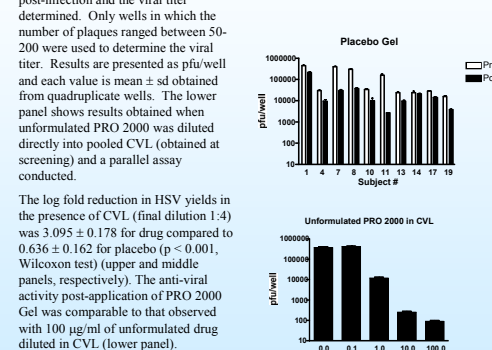


Figure 3: Anti-HSV activity of CVL obtained pre- and post-application of PRO 2000 Gel (upper panel) or Placebo Gel (middle panel). Cells were pre-treated with CVL that had been diluted 1:2 in serum-free media for 15 min prior to being inoculated with serial dilutions of HSV-2; plaques were counted 48 h post-infection and the viral titer determined. Only wells in which the number of plaques ranged between 50-200 were used to determine the viral titer. Results are presented as pfu/well and each value is mean \pm sd obtained from quadruplicate wells. The lower panel shows results obtained when unformulated PRO 2000 was diluted directly into pooled CVL (obtained at screening) and a parallel assay conducted. The log fold reduction in HSV yields in the presence of CVL (final dilution 1:4) was 3.095 \pm 0.178 for drug compared to 0.636 \pm 0.162 for placebo ($p < 0.001$, Wilcoxon test) (upper and middle panels, respectively). The anti-viral activity post-application of PRO 2000 Gel was comparable to that observed with 100 µg/ml of unformulated drug diluted in CVL (lower panel).

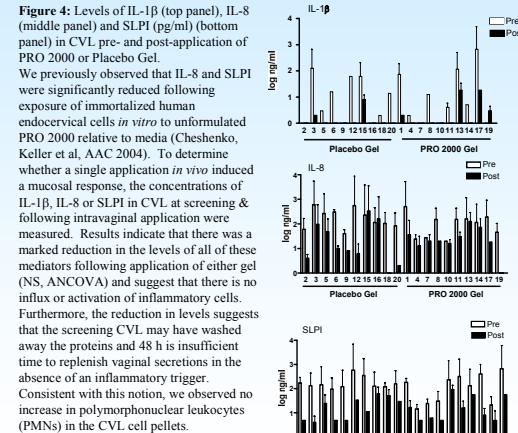


Figure 4: Levels of IL-1 β (top panel), IL-8 (middle panel) and SLP1 (pg/ml) (bottom panel) in CVL pre- and post-application of PRO 2000 or Placebo Gel. We previously observed that IL-8 and SLP1 were significantly reduced following exposure of immortalized human endocervical cells *in vitro* to unformulated PRO 2000 relative to media (Cheshenko, Keller et al, AAC 2004). To determine whether a single application *in vivo* induced a mucosal response, the concentrations of IL-1 β , IL-8 or SLP1 in CVL at screening & following intravaginal application were measured. Results indicate that there was a marked reduction in the levels of all of these mediators following application of either gel (NS, ANCOVA) and suggest that there is no influx or activation of inflammatory cells. Furthermore, the reduction in levels suggests that the screening CVL may have washed away the proteins and 48 h is insufficient time to replenish vaginal secretions in the absence of an inflammatory trigger. Consistent with this notion, we observed no increase in polymorphonuclear leukocytes (PMNs) in the CVL cell pellets.

Conclusions

- This study demonstrates for the first time that a candidate microbicide is sufficiently bioavailable and retains substantial anti-viral activity after intravaginal application.
- No differences in IL-1 β , IL-8 or SLP1 were observed following application of active compared to placebo gel.
- Testing additional compounds in the presently planned Phase II/III trials would allow validation of this approach and could determine whether this safe and inexpensive strategy provides a surrogate marker to predict efficacy. If confirmed, this strategy could be used to determine what future generation microbicides should go forward, which could result in major cost savings.

Future Studies

- Studies to assess the impact of seminal fluid on microbicidal activity are ongoing.
- 14-day studies of HIV-negative and HIV-positive women will more rigorously assess the impact of repeated applications of 0.5% PRO 2000 Gel compared to Placebo Gel on mediators of mucosal immunity.

Acknowledgements

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