CVLs from Women Vaginally Administered PC-1005 Inhibit HIV-1 and HSV-2 in the Mucosa

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BACKGROUND

- The Population Council's microbicide gel (PC-1005) containing 50µM MIV-150 (M), 14mM Zinc acetate dihydrate and carrageenan (CG) protect macaques against single high dose SHIV-RT challenge vaginally for up to 8h (1, 2), significantly reduces HSV-2 shedding in macaques (3) and HSV-2 and HPV infections in murine models (2).
- A recent Phase 1 trial demonstrated that PC-1005 gel applied vaginally once daily for up to 14 days is safe and well tolerated (4, 5).
- Cervico-vaginal lavage samples (CVLs) collected 4h or 24h after last gel application showed MIV-150 and CG dose-dependent inhibition of HIV-1 and HPV, respectively, in cell-based assays (4, 5).
- This pharmacodynamics study aimed to test activity of CVLs collected 4h or 24h after last gel application against HIV only and HIV-1/HSV-2 co-infection in human cervical mucosa.

RESULTS

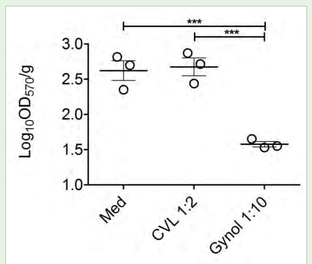


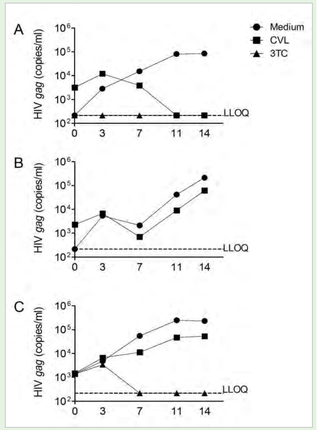
Figure 1 - Diluted CVLs do not decrease tissue viability.

Baseline CVLs were collected from participants in the Phase I PC-1005 trial before gel application using 10ml of saline and pooled. Viability of ectocervical tissue after immersion in medium containing a 1:2 dilution of CVLs (three explants per condition) for ~18h was tested by MTT assay (OD_{570} of the formazan product were measured and normalized by the dry weight of the explants). Each symbol indicates an individual donor and the Mean±SEM of the $Log_{10}OD_{570}$/g of tissue for each condition is shown. Log-normal generalized linear mixed models were used for statistical analysis. Significant p-values of <0.001 (***) are indicated.

RESULTS (CON'T)

Figure 2 - CVLs inhibit tissue infection with HIV-1_{BaL} when present during viral challenge, but not in the pre-exposure settings.

CVLs were collected as in Fig. 1. **(A)** Non-stimulated ectocervical explants were challenged with 500 $TCID_{50}$ of HIV-1_{BaL} (three explants per condition) ~18h in the presence of 1:2 diluted CVLs, then washed and cultured for 14d. Alternatively, tissues were pre-incubated with CVLs for **(B)** 18h or **(C)** 4h before viral challenge and cultured as described in (A). Infection was monitored by one step HIV *gag* qRT-PCR using culture supernatants (individual replicate analysis). Shown are summaries (Mean values) of n= 2-3 experiments.



Sample ID	CG (µg/ml)	MIV-150 (ng/ml)
M1004 HEC (4h)	ND	ND
M1007 HEC (4h)	ND	ND
M1023 HEC (24h)	ND	ND
M1001 PC-1005 (4h)	506.4	34.4
M1006 PC-1005 (4h)	403.5	51.0
M1008 PC-1005 (4h)	171.0	9.71
M1012 PC-1005 (4h)	817.4	36.3
M1014 PC-1005 (4h)	787.3	43.3
M1015 PC-1005 (4h)	973.1	59.9
M1016 PC-1005 (4h)	299.4	39.05
M1002 PC-1005 (24)	8.5	0.27
M1017 PC-1005 (24)	122.2	0.7665
M1019 PC-1005 (24)	ND	0.044
M1027 PC-1005 (24)	97.4	1.045
M1029 PC-1005 (24)	ND	0.000354
M1034 PC-1005 (24)	ND	0.1005

ND - not detected.
M1006 was excluded from the study due to contaminated CVLs.
M1012 and M1015 were excluded from HIV-1 (HIV-1_{BaL}/HSV-2 co-infection experiments) analysis due to lack of infection after pre-incubation with baseline CVLs.

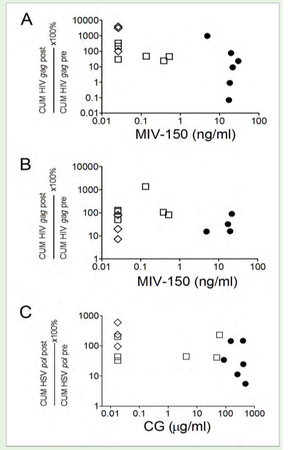
Table 1 - MIV-150 and CG concentrations in CVLs.

CVLs were available from 16 participants administered PC-1005 or HEC placebo gels vaginally daily for 14 days in the PC-1005 Phase 1 trial. CVLs were collected at the baseline and 4h (n=7 PC-1005, n=2 HEC) or 24h (n=6 PC-1005, n=1 HEC) post last gel administration. Concentrations of MIV-150 and CG in CVLs were measured by LC-MS/MS (LLOQ=100 pg/ml) and ELISA (LLOQ=58 ng/ml), respectively. No CG or MIV-150 were detected in the baseline samples.

RESULTS (CON'T)

Figure 3 - MIV-150 and CG in CVLs inhibit HIV and HSV-2 infection in the ectocervical explants in a dose-dependent manner.

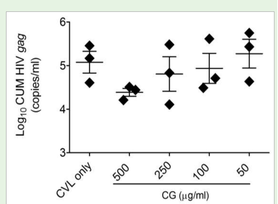
Tissues were incubated with paired 1:2 dilutions of CVLs (baseline and post gel) for 4h, washed and then challenged with 500 $TCID_{50}$ HIV-1_{Bal} or 500 $TCID_{50}$ HIV-1_{BaL} and 10^6 pfu HSV-2 per explant (three explants per condition) for ~18h. Following washout, tissues were cultured and infections analyzed by HIV *gag* qRT-PCR and HSV-2 *pol* qPCR in culture supernatants. CUM endpoint analysis was performed (6). Changes in CUM values after exposure to post gel CVLs ("post") vs. baseline CVLs ("pre") relative to MIV-150 (**A**, single challenge model; **B**, co-challenge model) and CG (**C**, co-challenge model) concentrations in diluted CVLs are shown (4h PC-1005 CVLs=solid circles, 24h PC-1005 CVLs= squares, placebo CVLs= diamonds).



- MIV-150 concentrations in CVLs inversely correlated with HIV-1_{BaL} infection in the tissues in the single challenge ($p<0.0001$) and co-challenge models ($p<0.05$). With every 10 ng/ml increase of MIV-150, HIV-1_{BaL} infection decreased by >60% and >30% in the single and co-challenge models, respectively. Infection inhibition was significant in the 4h (but not in 24h) post gel group vs. baseline ($p<0.01$) in the single challenge model. This was not the case in the co-challenge model.
- CG concentrations in CVLs inversely correlated with HSV-2 infection ($p<0.01$), resulting in ~30% decrease of HSV-2 infection per 100 µg/ml increase of CG. No significant differences between 4h and 24h groups vs. respective baselines were detected.

Figure 4 - CG partially inhibits HIV-1_{Bal} infection in ectocervical explants.

Baseline CVLs collected as in Fig. 1 were spiked with CG concentrations corresponding to concentrations in 1:2 diluted CVLs post PC-1005 application. Tissues were preincubated with CVLs+CG for 4h vs. CVLs only (three explants per condition), washed, challenged with HIV-1_{Bal} and cultured as in Fig. 3. Infection was monitored as in Fig. 3. Shown is CUM (Mean±SEM, n=3 experiments)



RESULTS (CON'T)

- CG (250-500 µg/ml) spiked into CVLs inhibited HIV infection by 55-83%. However, diluted CVL samples containing ~250-500 µg/ml of CG and ~17-30 ng/ml of MIV-150 showed higher anti-HIV-1 activity (~77-100% inhibition), pointing to MIV-150-mediated protection.

CONCLUSION

- ✓ Infections levels in the explants inversely correlated with MIV-150 concentrations (HIV-1) and CG (HSV-2) in the CVLs.
- ✓ Overall, our data demonstrate the potent anti-HIV and anti-HSV-2 activity of PC-1005 in mucosal targets and endorse the further development of PC-1005 as a broad-spectrum on-demand microbicide.

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FOR MORE INFORMATION

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