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# Carrageenan-Based Gel Retains Limited Anti-HIV-1 Activity 8–24 Hours After Vaginal Application by HIV-Infected Thai Women Enrolled in a Phase I Safety Trial

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## To the Editors

There is a need to improve in vitro testing to evaluate topical microbicide candidates that prevent acquisition of HIV. Many vaginal microbicides with different anti-HIV activities have undergone preclinical testing and a few of those have been selected for clinical safety and efficacy testing. Clinical efficacy trials of vaginal microbicide gels have yielded mixed results. Initial nonspecific entry inhibitors were shown to be ineffective in clinical efficacy trials,[1–4] whereas more recent testing of microbicide gels containing the nucleoside reverse transcriptase inhibitor, tenofovir, has shown some level of protection in 1 of 2 clinical trials.[5,6] Yet, nearly all preclinical testing outcomes predicted that products should significantly reduce sexual acquisition of HIV when used appropriately in a clinical trial. However, a recent report using ex vivo testing of the microbicide Pro2000 demonstrated that this product loses anti-HIV activity after vaginal application and sexual intercourse.[7] Changes in anti-HIV activity over time after vaginal application have not been studied for most candidate vaginal microbicides.

We investigated the carrageenan-based vaginal gel Carraguard, an HIV entry inhibitor that failed in a clinical efficacy trial,[1] for degradation and loss of anti-infective activity after vaginal application. Cervicovaginal lavages (CVLs) were collected from 16 HIV-infected Thai women participating in a randomized, controlled, 3-treatment (Carraguard, methylcellulose placebo, no product) crossover safety trial.[8] Women applied each treatment daily for 7 days after menses. The order of the 3 treatments was randomized. CVLs (5 mL) were collected on the first clinic visit in each treatment cycle before gel application ($T_0$), 15

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minutes after gel application ($T_{15min}$), and on day 7 clinic visit which was 8–24 hours after the final gel application ($T_{8–24hr}$). Self-reported adherence was 98% overall, and participants reported that vaginal application of the gel was highly acceptable.[9]

Carrageenan, the active ingredient of Carraguard, was measured in CVLs using a modified methylene blue assay.[10] Each CVL was diluted 1:26 in distilled water, mixed 10:1 with a 0.4-mM methylene blue aqueous solution, and its $OD_{540nm}$ was compared with those in a standard curve of carrageenan dilutions (lower limit of detection, 25 μg/mL). Carrageenan was detected in CVLs from all 16 women at $T_{15min}$ and from 12 women at $T_{8–24hr}$. Carrageenan levels in CVLs were significantly higher at $T_{15min}$ (median: 304 μg/mL; range, 103–3154 μg/mL) compared with $T_{8–24hr}$ (median: 98 μg/mL, range, <25–282 μg/mL) (Mann–Whitney, $P < 0.001$). Carrageenan was not detected in CVLs at $T_0$ nor in the CVLs collected after vaginal application of methylcellulose placebo or no product.

CVLs were then tested for carrageenan anti-infective activity using an ex vivo titer reduction assay. HIV-1 susceptible TZM-bl cells were plated in microtiter wells, briefly incubated in CVL supernatant, then virus-challenged with serial dilutions of a CCR5-tropic CRF01_AE HIV-1[11] using a standard infectious titer protocol[12] that reports 50% tissue culture infectious dose ($TCID_{50}$) values. The virus titer reduction value was the difference between $TCID_{50}$ from CVL samples at follow-up (T15min and $T_{8–24hr}$) and baseline ($T_0$). It was calculated for each woman and by type of treatment. The $T_0$ CVLs produced a narrow range of baseline $TCID_{50}$ values (4.67–5.55 $\log_{10}$, median 4.85 $\log_{10}$) that were slightly higher than those produced using normal cell culture media (median 4.67 $\log_{10}$, $P < 0.001$). All 16 T15min CVLs collected after Carraguard application produced a reduction in $TCID_{50}$ from 1 to >4.5 $\log_{10}$ and 12 of 16 CVLs at $T_{8–24hr}$ after Carraguard use reduced $TCID_{50}$ from 1 to 2.5 $\log_{10}$ (Fig. 1). Importantly, 3 of 4 CVLs with carrageenan levels more than 600 μg/mL at T15min completely prevented target cell HIV infection, and all 4 CVLs at $T_{8–24hr}$ with undetectable carrageenan levels showed no anti-infective activity. There was a strong correlation between a carrageenan level of CVL and its anti-infective activity (Spearman, $P < 0.001$, data not shown). In T15min and $T_{8–24hr}$ CVLs with similar carrageenan levels (100–300 μg/mL), there was no significant difference between their anti-infective activities in the titer reduction assay. CVLs collected after methylcellulose use (Fig. 1) had no measurable anti-infective activity. Native infectious virus was not detected in CVLs of the HIV-infected women, including those collected when women were using methylcellulose placebo or no product (data not shown).

Our ex vivo evaluation of anti-HIV activity indicates that Carraguard microbicide gel retained some ability to reduce HIV infection of target cells from 15 minutes up to 8–24 hours after vaginal application. Although carrageenan levels decreased in CVLs at $T_{8–24hr}$, their level of anti-infective activity ex vivo was not significantly different from CVLs at T15min with similar concentrations of carrageenan. Thus, the difference over time in anti-infective activity measured ex vivo was likely because of the reduction in recoverable carrageenan and not product inactivation in vivo. A similar result was reported for Pro2000 after vaginal application and sexual intercourse.[7]

The failure of Carraguard to prevent sexual acquisition of HIV-1 in a clinical efficacy trial was likely because of multiple factors including low adherence (41% of sex acts).[1] Nonetheless, data from our ex vivo assay suggests that carrageenan was present for at least 8–24 hours after vaginal application and retained some anti-infective activity. However, the reduction in genital tract carrageenan levels over time suggests that decreasing amounts of product at the cervical–vaginal mucosa might not have been sufficient to provide durable protection from virus in the semen of an infected male sexual partner. The carrageenan levels we measured produced only an infectious titer reduction, not complete protection from infection, in 13 of 16 CVLs at $T1_{5min}$ and in all 16 CVLs at $T_{8–24hr}$. In addition, the effectiveness of carrageenan-based microbicide gels in our ex vivo assay would likely be reduced further in the presence of semen as has previously been reported for other polyanion-based HIV-entry inhibitors.[13] Limitations of this study are the lack of controls for possible variations in how CVLs were collected and that semen was not included in our titer reduction assays. Our results show that vaginally applied carrageenan gel is not inactivated after 8–24 hours, but there is a significant loss of vaginal gel that would reduce the overall anti-infective capacity.

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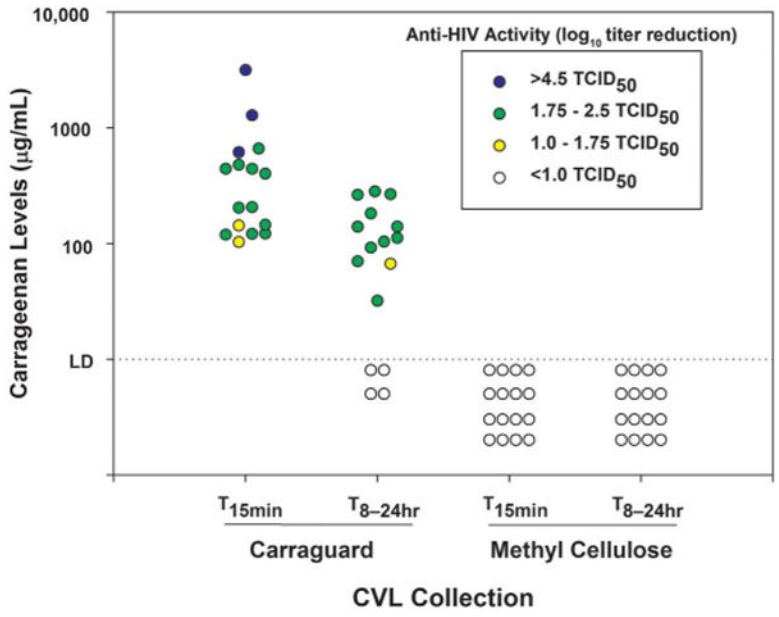


**FIGURE 1.**

CVLs containing carrageenan retain anti-HIV activity after exposure to the female genital tract. Carrageenan levels in CVLs were determined by a colorimetric methylene blue assay. Anti-HIV activity of each CVL was determined by virus titer reduction compared with $T_0$ CVL from the same woman and is represented by the colors in the symbol legend. LD, limit of detection for carrageenan in CVL (25 µg/mL).