

# Practical Prevention of Vaginal and Rectal Transmission of HIV by Adapting the Oral Defense: Use of Commercial Lubricants

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## ABSTRACT

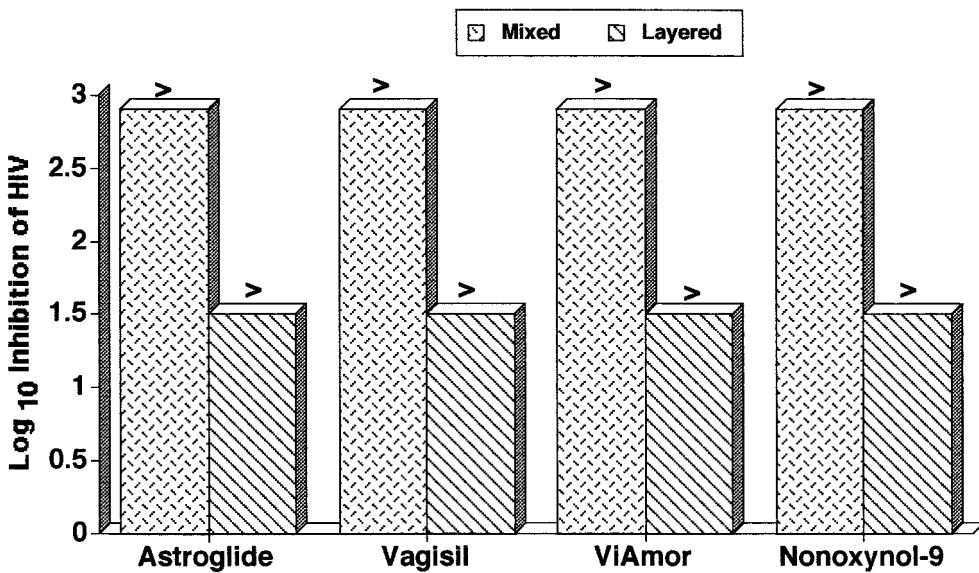
HIV is transmitted to 6.4 million human beings per year and the majority of these transmissions are sexual. Condoms are highly effective and are recommended as the primary preventive. However, the fact that there are millions of sexual transmissions each year indicates that many people do not use condoms and that additional preventives are needed. The mechanisms of natural prevention of oral transmission by saliva may be adaptable to the susceptible vagina and rectum. The objective of our study was to reduce the sexual transmission of HIV by mimicking saliva's targeting of the transmitting infected leukocytes and any cell-free HIV in seminal fluid. The previously recommended anti-HIV topical microbicide, nonoxynol-9, has not prevented HIV transmission in humans, probably because it causes mucosal irritation and attracts CD4<sup>+</sup> cells. To identify effective preparations that are nonirritating, we studied the anti-HIV activity of commercially available, over-the-counter (OTC) lubricants and vaginal preparations that are judged safest by the U.S. Food and Drug Administration (FDA), and are nonirritating. The effect of OTC preparations on both the production of HIV by infected leukocytes and cell-free HIV suspended in seminal fluid was measured under simulated *in vivo* conditions. We surveyed 22 OTC vaginal preparations and excluded those with low inhibitory activity and those that were inhibitory but likely to be irritating. Three included preparations are highly active against both HIV-infected leukocytes suspended in seminal fluid and active against cell-free HIV, under *in vitro* conditions that simulate *in vivo* conditions. Since the preparations identified here as anti-HIV substances have the advantages of being widely available, inexpensive, acceptable, in the safest U.S. FDA category, and may be used by recipient women or men, they should be tested in clinical trials to help prevent sexual transmission of HIV.

## INTRODUCTION

WORLDWIDE THERE ARE 34 million carriers of HIV. Transmissions of HIV per year number 6.4 million and the majority of these transmissions are sexual.<sup>1-3</sup> The main transmitting component of seminal fluid may be infected leukocytes,<sup>4-15</sup> rather than cell-free HIV, as previously thought.<sup>16-20</sup> This conclusion on cellular transmission is supported by the following evidence: (1) most of the infectivity of HIV in the body fluids and secretions of carriers is found in infected leukocytes rather than as free infectious virus<sup>16-19</sup>; (2) infected leukocytes may directly infect epithelial cells on mucosal surfaces or penetrate the epithelial layer<sup>4-9,20</sup>; (3) in comparison, any cell-free infectious HIV on mucosal surfaces is only weakly infectious

for epithelial cells that lack the CD4 receptor for HIV<sup>4,10,11</sup>; (4) simian mucosals, which similarly lack the CD4 receptor *in vivo*, are 1000 times more resistant to cell-free simian immunodeficiency virus than is the simian bloodstream,<sup>11-13</sup> which contains many CD4-positive leukocytes; and (5) hypotonic saliva, which preferentially inhibits HIV production by infected leukocytes by 10,000-fold, appears to contribute to the rarity of oral transmission.<sup>14,15</sup> Thus, sexual transmission may be reduced by chemically targeting the transmitting infected leukocytes in addition to targeting any cell-free HIV in seminal fluid.

The previously used anti-HIV topical microbicide, nonoxynol-9, has not been effective in humans,<sup>21-23</sup> probably because it evokes mucosal inflammation. To identify an effective but nonirritating preparation, we studied commercial, over-the-



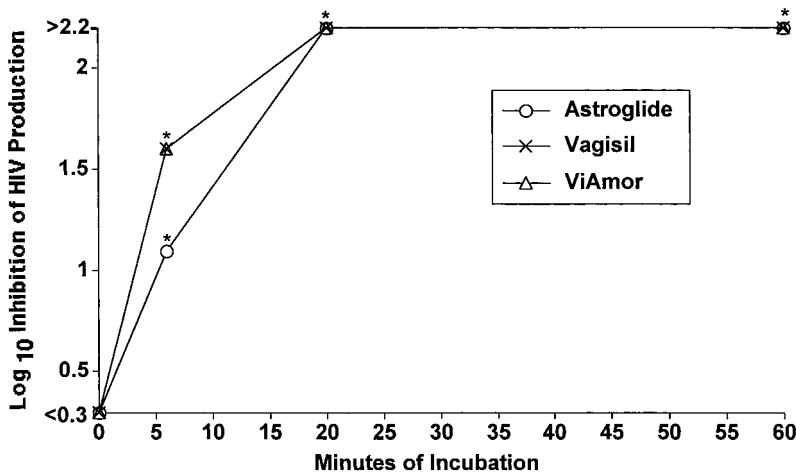
**FIG. 1.** Over-the-counter vaginal preparations inhibit HIV multiplication in CEM lymphocytes in seminal fluid compared with HIV multiplication in medium alone. Data are presented as log<sub>10</sub> inhibition in sample compared with medium control. The preparations are Astroglide vaginal lubricant, Vagisil vaginal moisturizer, and ViAmor vaginal moisturizer. The positive control is nonoxynol-9 (2.2%). All inhibitions are significant at  $p < 0.01$  by the Student  $t$  test (>, inhibition greater than the number indicated, compared with the medium controls).

counter (OTC) lubricants and vaginal preparations that are in the safest U.S. Food and Drug Administration (FDA) category and do not cause irritation when used repeatedly by large numbers of people.<sup>24</sup> We found three of these OTC lubricants to be active against HIV-infected leukocytes as well as against free HIV, suspended in seminal fluid. Therefore, they may be candidates for future clinical trials to prevent sexual transmission of HIV. Such OTC preparations have the additional advantages of wide availability, low cost, acceptance, and choice by women and men if their partners do not choose to use barrier prevention methods.

**MATERIALS AND METHODS**

*Antiviral OTC lubricants and vaginal preparations*

To identify OTC preparations that might inactivate the HIV-transmitting leukocytes, we purchased 22 topical OTC preparations and initially determined their activity against a surrogate virus (vesicular stomatitis virus) in cultured murine L cells by the method we reported previously.<sup>14</sup> Active preparations were then studied for their activity against HIV-infected lymphocytes or monocytes. The procedure used was either to mix or layer 150



**FIG. 2.** Time required for OTC vaginal preparations to inhibit production of HIV by infected CEM lymphocytes compared with production in medium alone.  $*p = <0.05$  to  $<0.01$  by the Student  $t$  test.

$\mu\text{l}$  of each OTC preparation over 150  $\mu\text{l}$  of seminal fluid containing  $6 \times 10^5$  CEM human lymphocytes or THP-1 monocytes, which had been infected with strain 213 HIV 4 days previously as described.<sup>14,15</sup> The mixture was then incubated for 1 hr at 37°C to permit inactivation of infected cells. The cells were then washed four times to remove extracellular virus, which was verified by assaying the residual HIV, and resuspended in RPMI medium containing 10% fetal bovine serum. The resuspended cells were incubated for 24 hr to allow production of new HIV virions. These 24-hr samples were titered for yield of infectious HIV, using four replicate 0.5 log<sub>10</sub> dilutions on MT-2 human lymphocytes.<sup>15,25</sup> The medium tissue culture infectious dose (TCID<sub>50</sub>) titer of virus yields was determined by microscopic reading of the cytopathic effect on day 5.<sup>25</sup> The titer was calculated by the Reed–Muench method.<sup>26</sup>

In addition to basic descriptive statistics described in the legends to Figs. 1–5, statistical methods such as regression analysis and nonparametric methods were used. In each instance, the pertinent trends and differences observed were large, and all related significance levels (*p* values) were small, even with the sample sizes used.

### Seminal fluid

Seminal fluids were collected from four normal men in accordance with our university Institutional Review Board guidelines. Samples were stored at 4°C for 1–2 days or, alternatively, stored at –20°C for up to 3 months. This storage was determined not to affect the properties of seminal fluid as assessed by permissiveness for HIV infection, absence of cytotoxicity, and maintenance of viscosity.

## RESULTS

### Inhibition of HIV production by various OTC vaginal preparations

Initially, the following OTC vaginal preparations were surveyed for inhibition of virus production by infected cells<sup>14,15</sup>: Astroglide Personal Lubricant, Betadine MD-Medicated Douche, Eckerd Extra Cleaning Vinegar and Water Douche, Eckerd Extra Mild Vinegar Douche, Femstat 3, Gyne-Lotrimin 3, KY Liquid-Personal Lubricant, KY Long Lasting Vaginal Moisturizer, KY Plus Spermicidal Lubricant (nonoxynol-9) 1%, Massengill Fresh Baby Powder Douche, Massengill Medicated Douche, Micronazole 7 Vaginal Cream, Mycelex-3, Refresh Disposable Extra Cleaning Formula Douche, Replens Vaginal Moisturizer, Silken Secret, Summer's Eve Extra Cleaning Vinegar and Water Douche, Summer's Eve Touch of Spring Douche, Summer's Eve Vinegar and Water Douche, Sweet Love Essence of Strawberry Douche, Vagisil Intimate Moisturizer, ViAmor Vaginal Moisturizer, Walgreen's Lubricating Jelly—Personal Lubricant 20%, and Walgreen's Natural Vinegar and Water Douche. Excluded from further study were preparations that had low inhibitory activity (<1.5 log<sub>10</sub> inhibition). Also excluded were preparations that were inhibitory but likely to be irritating (e.g., containing acids, Betadine, and nonoxynol-9) and, therefore, possibly enhancing HIV transmission.<sup>21,22</sup> Three OTC preparations, in the safest FDA category, met the criteria

and strongly inhibited HIV production by the infected human CEM lymphocytes. These preparations are Astroglide vaginal lubricant (Biofilm, Vista, CA), Vagisil vaginal moisturizer (Combe, White Plains, NY), and ViAmor vaginal moisturizer (Biofilm).

### Inhibition of HIV production by infected cells

As shown in Fig. 1, the three OTC preparations (Astroglide, Vagisil, and ViAmor) inhibited HIV production by >1000-fold when mixed with cells in seminal fluid, and >30-fold when layered on the cells in the two experiments shown in comparison with nonoxynol-9 (2.2%), which was used as a positive control. Similar inhibition occurred when using HIV-infected THP-1 human monocytes. The positive control, nonoxynol-9, gave similar inhibition. This inhibition may be attributed to decreased production of HIV by infected cells rather than to inactivation of released HIV by residual sample in the culture fluids because the infected cells were washed free of residual sample 1 hr after treatment. Therefore production of HIV over the next 23 hr occurred in the absence of sample (see Materials and Methods). Thus, three OTC lubricants and moisturizers strongly inhibit HIV production by HIV-infected human CEM lymphocytes and THP-1 monocytes suspended in seminal fluid.

To determine the time required for the OTC preparations to inhibit HIV production, the experiment was repeated with the layering of the OTC preparations for varying times from 5 to 60 min. The cultures were then washed before incubation for virus production. As shown in Fig. 2, at 5 min there was 10-

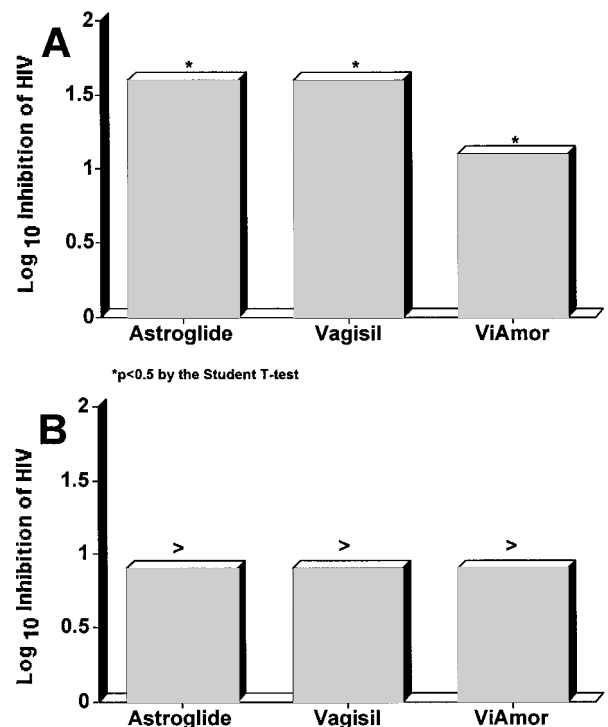
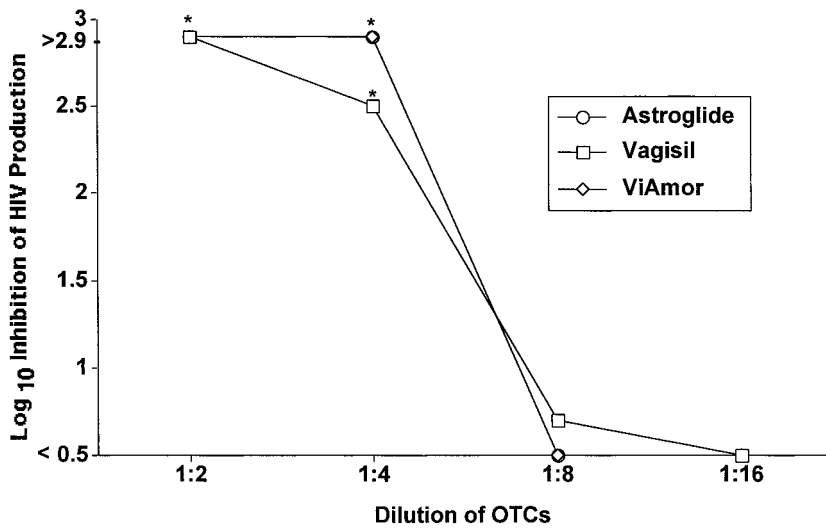


FIG. 3. (A and B) Over-the-counter vaginal preparations inhibit cell-free HIV in (A) medium or (B) seminal fluid. \**p* < 0.05 by the Student *t* test (>, inhibition greater than the number indicated, compared with the medium controls).



**FIG. 4.** Inhibitory titer of three OTC lubricants against HIV-infected human CEM lymphocytes in seminal fluid. \* $p < 0.01$  by the Student  $t$  test.

to 30-fold inhibition that increased to >160-fold inhibition at 20 and 60 min. Thus, rapid inhibition of HIV production occurs when the three OTC preparations are layered onto HIV-infected human CEM lymphocytes suspended in seminal fluid.

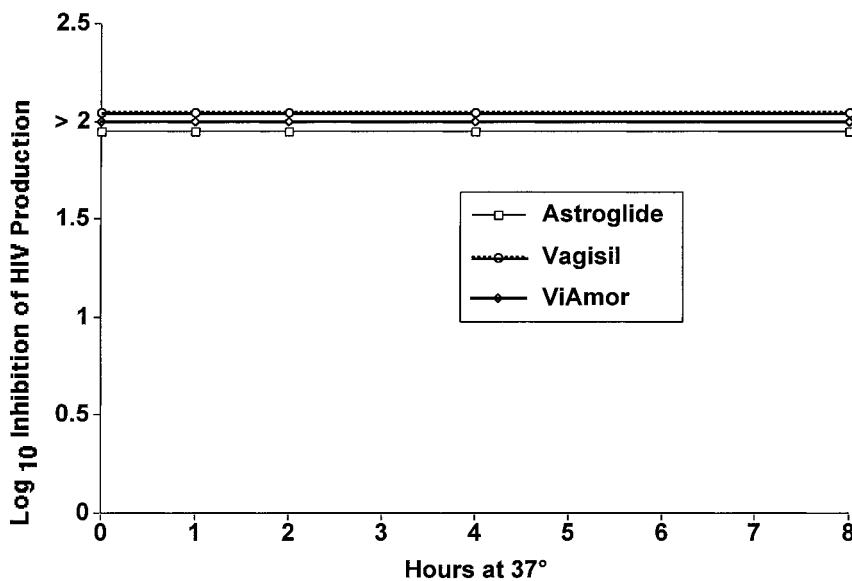
*Inactivation of cell-free HIV*

To determine whether cell-free HIV is inactivated by the OTC preparations we layered 200  $\mu$ l of the OTC preparation onto 200  $\mu$ l of seminal fluid containing  $1.6 \times 10^4$  TCID<sub>50</sub> of cell-free HIV and incubated for 1 hr at 37°C before titering for residual cell-free HIV infectivity. As shown in Fig. 3A and B, the three OTC preparations inhibited cell-free virus by about

10- to 30-fold in the absence of seminal fluid and 8-fold in the presence of seminal fluid. Thus, the OTC preparations can strongly inhibit cell-free HIV as well as inhibit infected lymphocytes.

To rule out a mechanical trapping effect or a pH effect as the main inhibitory mechanism, controls were thoroughly mixed or neutralized, and the same inhibition occurred. Specifically, neutralized preparations still inhibited HIV production and free HIV by  $\geq 30$ -fold. Also, mixed preparations inhibited HIV production or free HIV by  $\geq 30$ -fold. To ensure thorough mixing in the assays of HIV titers, the serial dilutions were fully solubilized.

To determine whether the OTC preparations would retain ac-



**FIG. 5.** Stability at 37°C of three OTC lubricants against HIV-infected human CEM lymphocytes in seminal fluid. All inhibitions are significant at  $p < 0.01$  by the Student  $t$  test.

tivity after dilution, we serially diluted them in culture medium and determined the activity of each dilution against HIV-infected CEM lymphocytes, using the methods described above. As shown in Fig. 4, at 8-fold dilution, the products lose effectiveness against infected CEM lymphocytes in seminal fluid. Thus the OTC preparations retain activity through a 1:4 dilution.

To be effective in people, the protective effect of the OTC preparations should be durable at 37°C for several hours. To determine the durability of the anti-HIV action against HIV-infected lymphocytes, the preparations were first incubated at 37°C for 1, 2, 4, and 8 hr and then layered onto infected cells in seminal fluid. As shown in Fig. 5, the >100-fold protection persisted at its maximum through 8 hr of incubation at 37°C. Thus, *in vitro*, the protective effect of the OTC preparations was durable through at least 8 hr at 37°C.

## DISCUSSION

There are 6.4 million HIV transmissions each year—the majority of which occur sexually.<sup>1–3</sup> Condoms are highly effective and are recommended as the primary preventive.<sup>27</sup> However, the occurrence of millions of sexual transmissions each year indicates that many people are not using condoms. Consequently, there is urgent need for additional practical methods to curtail sexual spread in underdeveloped as well as developed countries. An opportunity to develop such preventive measures comes from the recent understanding that the main transmitting entity in seminal fluid may be HIV-infected leukocytes rather than cell-free HIV. Such preferential targeting of infected leukocytes over cell-free HIV by saliva may account for the natural rarity of oral transmission.<sup>14</sup> Thus, prevention by targeting infected leukocytes, as well as any cell-free HIV, may reduce sexual transmission.

Considering the irritating effect of nonoxynol-9 OTC preparations, the present study attempted to identify effective but nonirritating preparations that would mimic protection by saliva. We studied commercial, over-the-counter (OTC) lubricants and vaginal preparations that are in wide use and in the safest U.S. FDA category I in the manner most often used throughout the world.<sup>24</sup> We found three of these OTC lubricants to be highly active against HIV-infected leukocytes and also effective against cell-free HIV. We also found that the inactivation of HIV-infected CEM lymphocytes was rapid (beginning at 5 min), and, therefore, early enough to inactivate infected lymphocytes prior to the 30 min required by them to transmit HIV to epithelial cells *in vitro*.<sup>5</sup> In addition, we found that the protective action of the OTC preparations was durable for more than 8 hr at 37°C *in vitro*.

It also is important that the OTC preparations retain their protective action in the vagina or rectum even after dilution by seminal fluid. Since the OTC preparations retained inhibitory activity against HIV-infected human CEM lymphocytes at a 1:4 dilution, we may estimate that a minimum volume of about 1 ml of the OTC preparation should be active *in vivo* against the average 3-ml volume of seminal fluid. A greater margin of safety would probably be provided by use of OTC volumes of greater than 1 ml (e.g., 3–5 ml), which are commonly used. Single-dose dispensers of 5 ml are marketed.

Also important is whether the protective activity would diffuse into the seminal fluid. To this end, comparison of layering versus mixing of the preparations with seminal fluid (Fig. 1) showed high protective activity of the preparations layered over lymphocytes in seminal fluid. This finding indicates that the protective activity of the preparations can diffuse into seminal fluid.

We are attempting to identify the active components of the OTC preparations and their mechanism of action. Inhibition, by the preparations, of HIV production by infected cells may be due to alteration of cellular functions or to disruption of infected cells. Analogous inhibition of HIV production occurs when infected human peripheral blood mononuclear leukocytes, lymphocytes, and macrophages are perturbed or disrupted by saliva and by nonisotonic culture fluids.<sup>14,15</sup>

Inhibition by the preparation of cell-free HIV is being studied. A possible mechanism for the direct inactivation of virions may be interaction with the envelope of HIV by surfactants in the formulations. Analogously, the surfactant nonoxynol-9 inactivates HIV virions.<sup>21</sup>

Overall the three OTC preparations have desirable properties, which should make them candidates for trials in humans. The attributes of these OTC preparations include being widely available, inexpensive, acceptable, in the safest U.S. FDA category, and usable by recipient women and men. We believe that the World Health Organization, government agencies, and pharmaceutical companies should strongly consider field trials in people at risk.

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