Inactivation of Human Immunodeficiency Virus Type 1 in Tissue Culture Fluid and in Genital Secretions by the Spermicide Benzalkonium Chloride

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Received 16 June 1989/ Accepted 11 September 1989

We have shown that the spermicidal agent benzalkonium chloride can exert a direct inhibitory effect on the viral reverse transcriptase activity of human immunodeficiency virus type 1 (HIV-1) when utilized at concentrations of 0.05% and higher. Exposure of HIV-1 to this disinfectant at concentrations of more than 0.05% was able to completely destroy viral infectivity, as assessed on susceptible target cells. We have further shown that HIV-1, which is present in both seminal and genital secretions, can be inactivated in such fluids by direct exposure to benzalkonium chloride.

We have questioned whether the spermicidal agent benzalkonium chloride (BC) possesses the ability to inactivate the human immunodeficiency virus type 1 (HIV-1), the etiologic agent of acquired immune deficiency syndrome (1, 5). Previous data have shown that this compound is an effective spermicidal agent yet is nontoxic for skin and other bodily surfaces with which it may come into contact (3, 4, 8). Indeed, BC has been employed in both contraceptive sponges and condoms as an effective spermicidal ingredient (3, 8). Other spermicides have, of course, also been used for this purpose; most notably, nonoxynol-9 has been shown to kill HIV-1 (9).

Our study focused on whether BC could directly destroy or inactivate viral reverse transcriptase activity, upon contact with virus, and what the effect of such treatment might be on the ability of HIV-1 to infect and to produce infectious virus (1.5 × 104 PFU/ml of HIV-1, as assessed on susceptible MT-4 cells (7)), were exposed to a variety of concentrations of BC in RPMI 1640 medium for 10 min at room temperature. After this procedure, virus suspensions were pelleted by ultracentrifugation and suspended in a preparation of erythrocyte ghosts in RPMI 1640 medium for 10 min to neutralize any potentially toxic effects of residual drug on cells in tissue culture. The erythrocyte ghosts employed were from the same individuals who served as donors of peripheral blood lymphocytes (PBLs) used as targets in HIV infectivity assays. Toward this end, disinfectant-treated preparations of HIV were suspended in 0.1 ml of erythrocyte ghosts that had been derived from the hypotonic lysis of 4.5 × 108 erythrocytes in 10 μM NaHPO4. We employed 1.5 × 106 PBLs as targets of HIV-1 infection, harvested by Ficoll-Isopaque centrifugation (2) from whole blood and suspended in 1 ml of growth medium.

These cultures were exposed to both untreated and treated preparations of HIV for 2 h in the presence of Polybrene (2 μg/ml) and were then monitored for production of viral reverse transcriptase activity and for the presence of viral p24 antigen. For purposes of reverse transcriptase assays, 0.2 ml of culture fluid was pelleted and assessed for the presence of viral enzyme, by previously described procedures (6). Viral p24 was assessed by antigen capture enzyme-linked immunosorption assay (Du Pont Co., Wilmington, Del.).

Semen and vaginal secretions were obtained from each of four male and four female individuals suffering from acquired immune deficiency syndrome, by autostimulation and saline lavage, respectively. The cells present in these fluids were pelleted and suspended in a small volume (0.5 ml) of RPMI 1640 growth medium. One-quarter of such suspensions was subjected to two cycles of freezing and thawing to release any cell-associated virus which might be present. The presence of HIV-1 antigens in these fluids was established by an antigen detection assay for the presence of HIV-1 p24. The presence of infectious HIV-1 was confirmed by direct isolation of the virus, following cocultivation of the remaining suspended cells with PBLs, obtained from healthy donors, as targets.
In order to demonstrate the effect of BC on the infectiousness of HIV-1 in human seminal and vaginal secretions, these fluids were exposed to concentrations of the disinfectant which had previously been shown to be effective against HIV-1 in tissue culture fluids (0.05%). After various times, the cells present in the bodily fluids were centrifuged at low speed and resuspended in fresh medium. These cells were subsequently examined for the ability to infect PBLs by a procedure in which both these cells and donor PBLs were separately prestimulated with phytohemagglutinin (PHA-P, 1:500 dilution; Difco Laboratories, Detroit, Mich.) for 2 days and subsequently cocultured in supplemented RPMI 1640 medium (500,000 cells of each type in 1 ml) containing recombinant interleukin-2 (2%, vol/vol; Boehringer Mannheim Biochemicals, Dorval, Canada). After 3-day intervals, the cells were again washed and reincubated with fresh phytohemagglutinin-pretreated target PBLs from healthy donors, as described above. The cultures were maintained in this fashion for a total of 30 days or until they became positive for the presence of detectable progeny HIV-1, as determined by either reverse transcriptase or p24 antigen capture assay.

Reverse transcriptase activity. Direct exposure of thawed preparations of H-9-derived HIV-1 to BC for 10 min had a significant effect on levels of viral reverse transcriptase activity detected after the repelling and suspension of virus (Fig. 1). Inactivation of enzymatic function took place in a concentration-dependent fashion in each of two separate experiments. These data indicate that BC possesses the ability to directly interfere with viral reverse transcriptase activity. The basis for this phenomenon is not understood.

Infectivity studies. Initial preliminary results had shown that BC at high concentrations (0.08 and 0.05%) had a toxic effect on the cell systems utilized (i.e., H-9 cells), probably because of residual drug remaining attached to the viral surface. Elimination of these toxic effects was accomplished by means of the erythrocyte ghost method described above, involving the placement of pelleted, treated virus in a suspension of erythrocyte ghosts to neutralize any residual BC. Virus was then repelled and used for determining infectiousness for H-9 cells which were previously untreated by HIV-1.

Those cells which were infected by either untreated HIV-III B or HIV-III B that had been exposed to erythrocyte ghosts but not to BC became positive for production of viral p24 antigen and reverse transcriptase activity by 7 days after inoculation (Table 1). In contrast, exposure of cells to HIV-III B that had been treated with either 0.05 or 0.08% BC did not result in active viral replication over periods of 12 and 30 days, as measured by the production of viral reverse transcriptase activity and p24 antigen, respectively. The use of lower concentrations of BC (0.005 and 0.01%) was less effective.

Presence and inactivation of HIV-1 in genital secretions. As described above, the presence of HIV-1 in vaginal and seminal secretions from each of four men and four women seropositive for HIV-1 was confirmed by direct isolation of HIV-1 from such fluids onto PBLs (Table 2). In two of eight patients studied (patients 3 and 6), the presence of viral p24 antigen could be directly demonstrated in such fluids (results not shown). We were unable to recover virus from the seminal fluids of an additional 10 patients, for whom no results are presented.

Samples of the seminal and vaginal secretions from these eight patients were treated with 0.05% BC for periods of

<table>
<thead>
<tr>
<th>TABLE 1. Inactivation of HIV-1 infectiousness by BC</th>
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* Culture medium was changed 24 h before assay for both p24 and reverse transcriptase. Optical density determinations were made by testing 200 μl of culture fluid for the presence of p24 antigen, by means of an enzyme-linked immunosorbent antigen capture assay. The values were subsequently converted to nanograms per milliliter on the basis of a standard curve. Samples were considered positive for p24 antigen at a level of more than 0.5 ng/ml.

** Samples were considered positive for reverse transcriptase activity when results were greater than 2,000 cpm/ml.

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TABLE 2. Effect of 0.05% BC on infectiousness of HIV-1 in human genital secretions

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Level of p24 antigen (ng/ml)* after treatment for:</th>
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<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>1</td>
<td>Male</td>
<td>11.7</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>15.2</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>11.0</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
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</tr>
<tr>
<td>5</td>
<td>Female</td>
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<td>6</td>
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<td>8.5</td>
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<tr>
<td>7</td>
<td>Female</td>
<td>15.9</td>
</tr>
<tr>
<td>8</td>
<td>Female</td>
<td>10.4</td>
</tr>
</tbody>
</table>

* In PBL cultures after 20 days. Culture medium was changed 24 h prior to the assay for p24, as described in Table 1; footnote a. Samples were considered positive for p24 antigen at a level of more than 0.5 ng/ml.
between 0.5 and 10 min. After centrifugation, the cells in these fluids were placed in a suspension of erythrocyte ghosts, as described above, and were then recentrifuged, stimulated with phytohemagglutinin, and used in cocultivation with PBLs. Exposure to BC for a period of 5 min was necessary to eliminate the presence of detectable infectious HIV-1 in such fluids (Table 2). Treatment of virus-containing fluids for 2 min appeared to result in a significant but incomplete diminution of viral infectivity for PBLs. In contrast, exposure to BC for 0.5 min was without effect. These data indicate that a 0.05% concentration of BC, when applied ex vivo for periods of 5 min or more, can destroy the infectiousness of HIV-1 in the genital fluids of seropositive patients.

The data presented above indicate that BC may have usefulness against HIV-1. The demonstration of the inactivation of cell-associated HIV-1 in vaginal and seminal secretions complements the already-documented spermicidal properties of this series of compounds (3, 4, 8) and may have applicability to the prevention of sexually transmitted disease. In addition to its spermicidal properties, BC is used as a disinfectant in contact lens solutions (8).

This research was initiated because of the need to document the potential viricidal properties of substances like BC that are already employed in other areas (11). BC is thought to act as a spermicide at the surface of sperm cells to alter and reduce motility. Although its mechanism of action with regard to HIV-1 is unknown, it is likely that surface alterations of the virus may be important.

To date, only one other disinfectant has been used to inactivate HIV-1 under conditions of potential sexual transmission. This compound, nonoxynol-9, possesses anti-HIV-1 activity; it is used in condoms and vaginal suppositories to add an extra dimension of protection against the transmission of HIV-1 and other sexually transmitted agents (9). It is possible that some individuals may be unable to tolerate nonoxynol-9, for a variety of reasons, including allergic reactions in some people who are exposed to it on a regular basis. Thus, BC may be able to play a complementary role to nonoxynol-9 as an ingredient of spermicidal jellies, foams, and condoms.

This research was supported by a grant from Health and Welfare Canada.

We thank Normand Blain for excellent technical assistance and Francine Busschaert for the preparation of the manuscript. We also thank Laboratoire Bio-Chimique Inc. of Montreal, Quebec, Canada, for a gift of BC.

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