Recovery of Human Immunodeficiency Virus From Peritoneal Dialysis Effluent

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ABSTRACT

Increasing numbers of HIV-infected patients who have ESRD are being treated with continuous ambulatory peritoneal dialysis (CAPD). To investigate the potential infectious nature of peritoneal dialysate (PD), the peritoneal dialysis effluent was studied in 14 patients on CAPD who were known to be HIV antibody positive. Peripheral blood mononuclear cells and the sediment of PD were obtained from each patient and subjected to a qualitative microculture assay using a coculture of patient cells or PD fluid with peripheral blood mononuclear cells from non-HIV-infected individuals. Samples from the coculture were collected twice weekly for HIV P24 antigen determination as a marker of viral replication. PD, white blood cell and red blood cell counts, and peripheral blood CD4 lymphocyte counts were also measured. All 14 patients developed a positive blood culture by Day 3. Twelve of the 14 patients developed a positive PD fluid culture. The mean CD4 count was 310 cells/mm³. No patient had clinical or cellular evidence of peritonitis at the time of fluid sampling. These data indicate that peritoneal dialysis effluent from patients who are HIV antibody positive is potentially infectious.

Key Words: HIV, peritoneal dialysis, AIDS, ESRD, dialysis

Infection with HIV has been reported in over 1 million cases in the United States and 17 million cases worldwide (1). Over 900,000 of these individuals have progressed to the acquired immunodeficiency syndrome (AIDS), with 42% of these cases occurring within the United States (2). Renal manifestation of HIV infection has been estimated to occur in 6 to 10% of these individuals, with 40% of these patients requiring renal replacement therapy (3–5).

Initial reports painted a bleak prognosis for these individuals on dialysis, and both hemodialysis and continuous ambulatory peritoneal dialysis (CAPD) were discouraged (5, 6). Recently, however, survival rates of between 14 and 18 months have been reported for both hemodialysis and peritoneal dialysis by multiple centers (7–9). The choice of modality between hemodialysis and peritoneal dialysis is widely debated and currently rests with physician preference.

Peritoneal dialysis offers several advantages over hemodialysis: (1) the lack of vascular access eliminates the extra corporeal blood circuit, which if interrupted, is potentially infectious to both staff and the surrounding patients; (2) CAPD may result in less stimulation of HIV-infected T cells because of the lack of stimulatory cytokines that may be produced secondary to the blood membrane interaction during hemodialysis (10); and (3) patients on CAPD have been reported to have improved humoral immunity compared with those patients on hemodialysis (11). Because of the aforementioned issues, many nephrologists have preferentially placed HIV-infected patients on peritoneal dialysis. At least two studies have shown peritoneal dialysis to be equally effective to hemodialysis in this patient population (7, 8).

One concern that remains regarding the use of peritoneal dialysis in HIV-infected patients is the potential infectious nature of the large volumes of dialysate that is generated. Initial investigation into the potential infectious nature of peritoneal dialysis effluent (PDE) in small numbers of patients has suggested that viable HIV can be recovered from PDE and is potentially infectious (12–14). In this study, we have analyzed HIV-1 recovery from PDE and peripheral blood of 14 patients who were serologically positive for the HIV antibody and who have been maintained on peritoneal dialysis at the Johns Hopkins Hospital between January 1, 1992, and July 1, 1994.

MATERIALS AND METHODS

Patients

From January 1, 1992, through July 1, 1994, 57 patients were placed on CAPD at the Johns Hopkins Hospital. Fourteen of those patients were known to be antibody positive for HIV. The mean age was 39.5 with a range of 29 to 55 yr old. Eleven (79%) of the 14 patients were men, and all patients were African-American. Table 1 indicates the cause of ESRD, sex, age, and race and documents whether or not the patients were taking AZT at the time of fluid sampling. All patients were trained for CAPD with the Ultrabag System (Baxter Health Care, McGaw Park, IL).
TABLE 1. Demographics of peritoneal dialysis HIV study population

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Race</th>
<th>Cause of ESRD</th>
<th>Patient Taking AZT?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>40</td>
<td>Black</td>
<td>GN</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>32</td>
<td>Black</td>
<td>GN</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>36</td>
<td>Black</td>
<td>GN</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>44</td>
<td>Black</td>
<td>GN</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>32</td>
<td>Black</td>
<td>HTN</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>41</td>
<td>Black</td>
<td>GN</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>29</td>
<td>Black</td>
<td>HTN</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>50</td>
<td>Black</td>
<td>GN</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>Male</td>
<td>52</td>
<td>Black</td>
<td>HTN</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>Male</td>
<td>55</td>
<td>Black</td>
<td>DM</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>Male</td>
<td>42</td>
<td>Black</td>
<td>HTN</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>Female</td>
<td>40</td>
<td>Black</td>
<td>GN</td>
<td>Yes</td>
</tr>
<tr>
<td>13</td>
<td>Male</td>
<td>33</td>
<td>Black</td>
<td>HTN</td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>Male</td>
<td>35</td>
<td>Black</td>
<td>GN</td>
<td>No</td>
</tr>
</tbody>
</table>

* GN, glomerulonephritis; HTN, hypertension; DM, diabetes mellitus.

PDE Cultures

Two liters of PDE were obtained from each patient, from a random exchange. All effluent contained 2.5% dextrose. The effluent was immediately centrifuged for 10 minutes at 400 g. The supernatant was discarded, and the cellular pellet was resuspended in a solution containing 1.0 × 10^6 peripheral blood mononuclear cells (PBMC) obtained from HIV-negative donors. The cocultivating cells were stimulated in RPMI culture media containing 20% fetal bovine serum, 2% interleukin-2, 1.0% sodium bicarbonate, 2 mM L-glutamine, and 3 μg/mL of PHA-P (Diffco, Detroit, MI) for at least 24 h. The cells were transferred to a conical flask and cultured at 37°C. Aliquots from the culture were collected twice weekly for 4 wk and assayed for the HIV P24 antigen by an ELISA technique (Abbott Laboratories, North Chicago, IL). Viral replication was considered present if >30 pg/μL of HIV P24 antigen was present, and serial analysis revealed rising quantities of the P24 antigen. All cultures were discarded at 28 days.

Blood Cultures

Ten milliliters of whole blood was collected from each patient in heparinized tubes. PBMC were isolated from heparinized blood by ficoll-hypaque density gradient. Patient PBMC were cocultivated with 10 million HIV-negative PBMC, as described earlier (PDE culture). The cells were transferred to a conical flask and cultured at 37°C for 28 days. Aliquots from the culture were collected twice weekly and assayed for the HIV P24 antigen by an ELISA technique (Abbott Laboratories). Viral replication was considered present if culture supernatants revealed >30 pg/μL of P24 antigen and serial analysis revealed rising quantities of the P24 antigen.

PD Fluid Analysis

Red blood cell (RBC) and white blood cell (WBC) counts were obtained manually on unspun PDE from each specimen submitted for culture. The results of both RBC and WBC counts are expressed in cells per cubic millimeter.

CD4 Counts

Peripheral blood CD4 counts were obtained on all patients at the time of PD fluid sampling. CD4 counts were determined with anti-CD4 antibodies and standard flow cytometry (Coulter Corporation, Hialeah, FL).

RESULTS

The results of PD fluid cell count and culture, as well as blood culture and CD4 results, are presented in Table 2. Twelve of the 14 patients studied had positive PD fluid cultures for HIV-1 by Day 28 of culture. The remaining two patients remained negative. Five patients (36%) had developed positive cultures by Day 3, three (21%) by Day 6, two (14%) by Day 12, and one (7%) by Day 21. All patients had positive blood cultures for HIV-1.

Peripheral blood CD4 counts ranged from 5 to 761 cells/mm³, with the mean CD4 count being 310 cells/mm³. The mean CD4 count in patients with positive PD fluid cultures for HIV was 228 cells/mm³, and the mean CD4 count for those patients with negative PD fluid cultures was 570 cells/mm³.

No patient had chemical or clinical evidence of peritonitis at the time of PD fluid sampling. PD fluid RBC ranged from zero to three cells/mm³ with a mean of one RBC/mm³. Peritoneal dialysis fluid WBC counts ranged from zero to six WBCs/mm³ with a mean of one WBC/mm³.

DISCUSSION

The isolation of the antibody to HIV-1 from PDE was first reported in 1986 from a single patient (13). This initial report, although not conclusive, suggested that, like hepatitis B, PDE may be a reservoir of infectious virus (15, 16). Over the next several years, conflicting reports arose as to the potential infectious nature of this fluid. Breyer and Harbison described the recovery of HIV from the PD in two of three patients who were known to be serologically positive for antibody to HIV-1 (12). Similarly, Goffin and colleagues described the recovery of HIV from the PDE in a single patient using a coculture system (14). Finally, Kupferman et
TABLE 2. Results of HIV-1 isolation and blood cell counts of PDE and peripheral blood obtained from 14 HIV-1 serologically positive dialysis patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>CD4 Count (cells/mm³)</th>
<th>HIV Blood Culture</th>
<th>HIV PD Fluid Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>+</td>
<td>+Day 6</td>
</tr>
<tr>
<td>2</td>
<td>761</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>+</td>
<td>+Day 3</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>+</td>
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<tr>
<td>5</td>
<td>134</td>
<td>+</td>
<td>+Day 3</td>
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<tr>
<td>6</td>
<td>338</td>
<td>+</td>
<td>+Day 3</td>
</tr>
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<td>7</td>
<td>109</td>
<td>+</td>
<td>+Day 3</td>
</tr>
<tr>
<td>8</td>
<td>374</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>490</td>
<td>+</td>
<td>+Day 12</td>
</tr>
<tr>
<td>10</td>
<td>193</td>
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<td>+Day 6</td>
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<tr>
<td>11</td>
<td>510</td>
<td>+</td>
<td>+Day 21</td>
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<td>12</td>
<td>129</td>
<td>+</td>
<td>+Day 6</td>
</tr>
<tr>
<td>13</td>
<td>313</td>
<td>+</td>
<td>+Day 12</td>
</tr>
<tr>
<td>14</td>
<td>410</td>
<td>+</td>
<td>+Day 9</td>
</tr>
</tbody>
</table>

al. failed to demonstrate HIV recovery from the PD of one child undergoing automated peritoneal dialysis using the polymerase chain reaction (17).

The 14 patients described in this study represent the largest cohort report in which HIV recovery from PDE has been attempted. Our study indicates that HIV can be recovered from 86% of the patients that we tested and confirms previous reports that PDE from HIV-infected individuals is potentially infectious.

It is not possible by the study methods used in this report to determine the viral load of each PD exchange or the quantity of PD fluid required to potentially infect a health care worker or family member. HIV is known to exist intracellularly, and the viral load could be expected to be greater, with a larger cell fraction in the PD, as occurs with peritonitis (18).

Five patients with positive PDE cultures for HIV had no WBC detected on routine PDE sampling. It is possible that there were WBC present in PDE in numbers too low to be detected by the methods used in this study. Unfortunately, no cell counts were obtained on the pellet of PDE after centrifugation. A second possibility would be the presence of cell-free virus in PDE.

Two patients in our study had negative peritoneal dialysis cultures for HIV while having positive blood cultures for this virus. Possible explanations would include a smaller cellular fraction in these exchanges, lower HIV-1 viral load, and possibly, improved immunity in these patients associated with higher CD4 counts.

Fifty-seven percent of the patients in this study were taking AZT during the time of PD fluid sampling. Neither patient who had a negative PD fluid effluent culture was taking AZT. Because all patients receiving AZT had recoverable HIV-1 in their PD effluent, AZT does not seem to reduce the infectious nature of this fluid to the level below the sensitivity of HIV-1 isolation techniques.

We conclude that PDE in individuals infected with HIV is potentially infectious. Further study will be needed to determine the length of time that HIV remains viable in the PD fluid and the dialysate tubing. Different methods of disinfection should be investigated to assure the safe disposal of this material.

REFERENCES