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# Pharmacokinetics of Hyperimmune Anti-Human Immunodeficiency Virus Immunoglobulin in Persons with AIDS

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**Hyperimmune anti-human immunodeficiency virus immunoglobulin (HIVIG) is an intravenous immunoglobulin prepared from HIV-infected asymptomatic donors with a CD4 cell count greater than 400 cells/μl and a high titer of antibody to HIV-1 p24 protein. Twelve persons with AIDS received four doses of HIVIG (two at 50 mg/kg of body weight and then two at 200 mg/kg) every 28 days. Pharmacokinetics were evaluated by measurement of anti-p24 antibody. HIVIG was well tolerated, and all participants completed the study. Three subjects who were not receiving *Pneumocystis carinii* pneumonia (PCP) prophylaxis developed PCP. The mean value for HIVIG clearance was 3.02 ml/kg/day at 50 mg/kg and 3.65 ml/kg/day at 200 mg/kg ($P = 0.027$); the mean trough antibody titers (reciprocal units) were 1,442 and 4,428, respectively. This study indicates that high titers of anti-p24 antibody can be maintained with a monthly administration schedule of HIVIG and that short-term safety is acceptable. Comparisons to evaluate the therapeutic potential of HIVIG are justified.**

Immunoglobulin products have demonstrated therapeutic or prophylactic efficacy against several human pathogens, including cytomegalovirus, varicella-zoster virus, hepatitis B virus, rabies virus, and many bacterial species (10, 11, 15, 16). Administration of human immunodeficiency virus (HIV) hyperimmune globulin or plasma preparations to adults (6, 7) and one child (18) with AIDS indicated that these products reduced plasma viremia and led to some clinical improvement.

Hyperimmune anti-HIV immunoglobulin (HIVIG) is a hyperimmune anti-HIV intravenous immunoglobulin prepared from pooled plasma of multiple HIV-infected asymptomatic donors from geographically diverse regions of the United States. These donors have a CD4 lymphocyte count over 400 cells/μl, a high titer of antibody to HIV-1 p24 protein, and no detectable HIV antigen, and they are nonreactive for hepatitis-B surface antigen and antibody to hepatitis C virus (4). The final product has a high titer of anti-p24 antibody (>1:200,000), demonstrates HIV-1-neutralizing activity, and has high antibody binding to the third hypervariable domain (V3) loop of HIV-1 glycoprotein 120. In vitro, HIVIG demonstrated inhibition of syncytium formation, directed group-specific antibody-dependent cellular cytotoxicity against HIV-infected targets, and inhibited cytopathic effect in T cells. HIV replication in monocytes/macrophages was inhibited in vitro by HIVIG concentrations of 50 to 500 μg/ml (anti-p24 antibody reciprocal titer values of approximately 250 to 2,500). Administration of the product to two juvenile chimpanzees showed that the product was safe and did not transmit HIV and that the half-life of anti-p24 antibody was 11 to 17 days (4). The objective of this study was to evaluate the pharmacokinetics and safety of HIVIG following the first administration of the product to humans.

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## MATERIALS AND METHODS

**Patients.** Twelve persons with AIDS who had one or more prior episodes of histologically or cytologically proven *Pneumocystis carinii* pneumonia (PCP) and who had been tolerating zidovudine therapy (≥300 mg/day) for at least 90 days before enrollment were eligible to participate in this investigation. Six participants were required who had measurable HIV (p24) antigen in the serum (>25 pg/ml). Additional entry criteria included a Karnofsky performance index of 60 or greater, a granulocyte count over $1,500 \times 10^6$/liter, a platelet count over $100 \times 10^9$/liter, hemoglobin over 105 g/liter, serum creatinine less than 1.25 times the upper limit of the normal range, and alanine aminotransferase and aspartate aminotransferase less than four times the upper limit of the normal range. Criteria for exclusion from participation were active parenteral substance abuse, recent receipt of investigational anti-HIV compounds, absence of IgA, or active life-threatening HIV-related opportunistic infections.

This investigation was conducted in the Outpatient HIV Clinic and was approved by the Institutional Review Board at the University of Minnesota. All subjects were informed about the study, and written consent was obtained prior to participation.

**Drug administration and patient assessments.** HIVIG was prepared as described elsewhere (4). The final product was formulated as a 5% protein solution in normal saline. HIVIG was administered intravenously every 28 days; the first two doses were 50 mg/kg of body weight followed by two doses at 200 mg/kg. At entry, prior to each HIVIG infusion, and for two months after study completion, each participant received a physical examination and laboratory studies, including a complete blood count, differential leukocytes, T-lymphocytes, platelets, reticulocytes, serum electrolytes, renal and hepatic function studies, urinalysis, electrocardiogram, chest roentgenogram, and serum HIV antigen. Clinical toxicity was assessed with the schema of the AIDS Clinical Trials Group (ACTG).

**Pharmacokinetic evaluations.** Anti-p24 antibody was selected as the primary determinant of the pharmacokinetics of HIVIG. Blood samples for measurement of anti-p24 antibody were obtained before the first infusion of HIVIG and at 5 min, 1 and 6 h, and 1, 2, 7, 14, and 28 days after the end of infusion. Samples were similarly collected before and after each subsequent infusion with the exception of the 1- and 6-h and 14-day time points; an additional sample was collected at 56 days after the end of the fourth infusion. The selection of these sampling times was guided by a D-optimal sampling strategy (5). A set of four observation times (5 min and 1, 7, and 28 days) was selected based on the assumption that HIVIG would exhibit pharmacokinetic characteristics consistent with those of other intravenous immunoglobulins, i.e., biexponential decay and an average elimination half-life of 20 days (in healthy persons) (3, 17). These times were 87% of a fully D-optimal design after the first dose. The additional points at which samples were collected after the first dose were selected based on investigator judgment. Anti-p24 antibody was measured with a specific competitive enzyme immunoassay (Envacore; Abbott Laboratories, Delkenheim, Germany) (1). This assay had an interday coefficient of variation of 10% over a range of 1:9 to 1:30,000.

The reciprocal value of each anti-p24 antibody determination (reciprocal titer

TABLE 1. Patient characteristics

| Patient | Age (yr) | Mo post-PCP | $CD4^+$ cells/μl | | HIV antigen (pg/ml) | | AIDS events on HIVIG |
|---|---|---|---|---|---|---|---|
| | | | Start | Stop | Start | Stop | |
| 1 | 30 | 10 | 435 | 282 | 73 | Neg[a] | None |
| 2 | 33 | 7 | 7 | 12 | 246 | Neg | None |
| 3 | 36 | 9 | 11 | 21 | 792 | Neg | None |
| 4 | 25 | 12 | 10 | 14 | Neg | Neg | PCP |
| 5 | 35 | 28 | 15 | ND[b] | 39 | Neg | PCP |
| 6 | 36 | 14 | 10 | ND | Neg | Neg | None |
| 7 | 31 | 4 | 66 | 77 | Neg | Neg | None |
| 8 | 66 | 10 | 43 | 43 | Neg | Neg | None |
| 9 | 31 | 32 | 5 | 7 | 89 | Neg | PCP |
| 10 | 35 | 5 | 56 | 34 | Neg | Neg | None |
| 11 | 41 | 21 | 18 | 17 | 1,198 | Neg | None |
| 12 | 38 | 4 | 37 | 43 | Neg | Neg | KS[c] |

[a] Neg, negative.
[b] ND, not done.
[c] KS, Kaposi's sarcoma.

[RT]) was used for pharmacokinetic analysis. Concentration-time data for each patient and each infusion were graphically inspected. A series of compartmental models, beginning with a one-compartment first-order elimination model and progressing to two- and three-compartment models, were then fitted to the anti-p24 antibody concentration-time data with maximum likelihood estimation (ADAPT II; Biomedical Simulations Resource, University of Southern California, Los Angeles) (5). Models were formatted to accommodate repetitive, nonuniform infusions. Output error was modeled with a proportional variance approach. Selection of the final pharmacokinetic model was based on residual analysis and calculation of the Akaike information criterion. The pharmacokinetic parameters obtained for HIVIG were evaluated statistically to test for differences between the HIV antigen-positive and -negative patients and between the 50 mg/kg and 200 mg/kg dose and for any interaction between HIV antigen status and HIVIG dose. A repeated measures analysis of variance was used for this evaluation; a $P$ value of $<0.05$ defined statistical significance.

## RESULTS

Twelve HIV-infected men with AIDS were enrolled in this study. All completed the four infusions of HIVIG and the final scheduled clinic visit. Table 1 presents demographic data on these individuals. HIVIG was well tolerated by these 12 men. No laboratory toxicities were found. One participant (number 7) experienced a 20-min episode of lightheadedness beginning approximately 45 min after the third infusion. This study was performed before routine prophylaxis for PCP had been established as standard care. Therefore, unfortunately, three subjects who were not receiving PCP prophylaxis developed PCP during the 6 months between the first infusion of HIVIG and the final evaluation.

Pharmacokinetic parameters for HIVIG are presented in Table 2. All participants had pretherapy anti-p24 antibody titers that were ≤1:10. Anti-p24 antibody concentrations following the administration of HIVIG were generally best described with a two-compartment pharmacokinetic model; concentration data for two patients at the 50 mg/kg dose level were best characterized with a one-compartment model. The mean elimination half-life ($T_{1/2\beta}$) was 21.3 days for the 50 mg/kg dose and 18.9 days at 200 mg/kg. The area under the curve (AUC) averaged 102,739 RT · day for the 50 mg/kg dose and was 356,414 at 200 mg/kg. The mean value for total body clearance (CL) of anti-p24 antibody was 3.02 ml/kg/day at 50 mg/kg and 3.65 ml/kg/day at 200 mg/kg. In HIV antigen-positive individuals, compared with HIV antigen-negative individuals, $T_{1/2\beta}$ was shorter ($P = 0.019$), AUC was smaller ($P = 0.04$), and CL was significantly higher ($P = 0.035$). Distribution volumes did not differ between HIV antigen-positive and -negative persons. The CL of HIVIG was significantly higher following a dose of 200 mg/kg than after a dose of 50 mg/kg ($P = 0.027$). There was a significant interaction between HIV antigen status, the dose of HIVIG, and antibody CL ($P = 0.019$) in that the increase in CL seen with the dose of 200 mg/kg was the result of an increased CL in the HIV antigen-negative persons. There was no correlation between the baseline concentration of HIV antigen in antigen-positive patients and antibody CL ($P > 0.5$). Figure 1 presents the mean anti-p24 antibody concentrations in the HIV antigen-positive and -negative patients.

## DISCUSSION

The administration of HIVIG to these 12 persons with AIDS produced high titers of anti-p24 antibody. The pharmacokinetic characteristics of this anti-p24 antibody include a steady-state distribution volume of approximately 80 ml/kg, biexponential decay with a terminal half-life of 20 days, and CL of 3.6 ml/kg/day. These characteristics are generally consistent with those of other immunoglobulin products (3). Furthermore, HIVIG was safe and well tolerated by the participants in this trial. There were no dose- or drug-limiting laboratory or clinical adverse events.

Six participants had measurable HIV antigen in the serum at entry into this study. Following the first dose of HIVIG, serum HIV antigen became undetectable and remained so throughout the remainder of the study. The presence of HIV antigen-

TABLE 2. HIVIG pharmacokinetic parameters[a]

| Dose and patients | $V_{ss}$[b] (ml/kg) | $T_{1/2\alpha}$[c] (days) | $T_{1/2\beta}$[d] (days) | CL (ml/kg/day) | AUC (RT · d) | Anti-p24 antibody[e] | |
|---|---|---|---|---|---|---|---|
| | | | | | | Peak | Trough |
| 50 mg/kg | | | | | | | |
| Antigen positive | 89.5 (28) | 0.63 (69) | 15.3 (17) | 4.18 (29) | 67,684 (40) | 5,940 (61) | 863 (22) |
| Antigen negative | 63.9 (42) | 1.63 (48) | 27.2 (37) | 1.86 (41) | 137,794 (28) | 9,980 (38) | 2,022 (29) |
| All patients | 76.7 (37) | 1.23 (67) | 21.3 (44) | 3.02 (51) | 102,739 (47) | 7,960 (52) | 1,442 (52) |
| 200 mg/kg | | | | | | | |
| Antigen positive | 92.8 (18) | 1.26 (97) | 17.7 (22) | 4.13 (32) | 271,949 (23) | 24,600 (43) | 3,298 (28) |
| Antigen negative | 75.4 (40) | 1.65 (90) | 20.2 (31) | 3.18 (49) | 440,880 (49) | 34,824 (44) | 5,557 (47) |
| All patients | 84.1 (30) | 1.46 (90) | 18.9 (27) | 3.65 (40) | 356,414 (49) | 29,712 (46) | 4,428 (50) |

[a] Values are expressed as means and coefficients of variation.
[b] $V_{ss}$, steady-state distribution volume.
[c] $T_{1/2\alpha}$, distribution phase half-life.
[d] $T_{1/2\beta}$, elimination phase half-life.
[e] Peak titers were obtained 5 min after the end of infusion; trough values were obtained 28 days after the end of infusion.

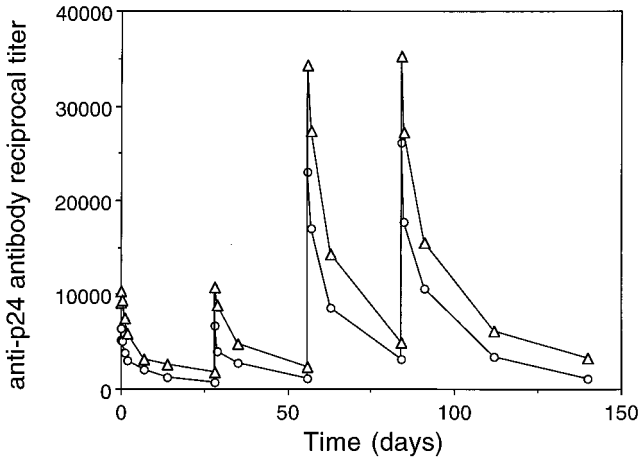


FIG. 1. Anti-p24 antibody RT values for antigen-positive and antigen-negative participants after two HIVIG doses of 50 mg/kg followed by two doses of 200 mg/kg. Each point represents the mean at the observation time in the six antigen-positive (circle) and six antigen-negative (triangle) individuals.

emia had a pronounced influence on the pharmacokinetics of HIVIG. The terminal half-life was shorter, AUC was smaller, and CL was higher compared with those of HIV antigen-negative persons. Presumably, the anti-p24 antibody delivered in the HIVIG preparation binds with HIV antigen in the serum (mostly p24 antigen) and this is responsible for an early, accelerated loss of antibody. The terminal half-life increased, although CL remained unchanged, after the dose of HIVIG was increased from 50 to 200 mg/kg for the six HIV antigen-positive persons. In contrast, the half-life decreased from 27.2 to 20.2 days and CL increased from 1.86 to 3.18 ml/kg/day for the HIV antigen-negative participants as their HIVIG dose was increased. These seemingly contradictory findings are consistent with two characteristics of immunoglobulin preparations: an increase in half-life as less antibody is lost through binding to infectious agents and a direct, proportional relationship between the catabolic rate and the concentration of immunoglobulin in the plasma (9, 17).

By analogy with the demonstrated efficacy of immunoglobulins for treatment and prevention of certain infectious diseases, HIVIG may have similar potential for HIV infection. The loss of anti-p24 antibody has been shown to have a temporal relationship with the appearance of HIV antigenemia and the progression of HIV disease (2). It is conceivable, then, that HIV disease might be delayed by maintenance of high anti-p24 antibody titers with HIVIG. Several factors have been identified as protective against maternal-to-fetal transmission of HIV. These include high levels and/or specific types of maternal antibodies directed against HIV. For example, pregnant women with antibodies to the V3 loop of glycoprotein 120 in one study were reported to have lower rates of transmission (13). This has not been a universal finding, however (12). Nontransmitting mothers have also been observed to more often have neutralizing antibodies against their own virus (14). Therefore, immunotherapy with HIVIG may be able to reduce maternal-fetal HIV transmission by decreasing infectious virus and/or providing antibodies with HIV-neutralizing activity.

This study of HIVIG indicates that high titers of anti-p24 antibody can be maintained with a monthly administration schedule and that the short-term safety and tolerance of the product is acceptable. These data justify evaluation of the therapeutic potential of HIVIG. Two controlled studies of HIVIG by the Pediatric ACTG are now under way. ACTG 185 is a multicenter, randomized, double-blind, controlled trial of HIVIG versus intravenous immunoglobulin for the prevention of maternal-infant HIV transmission in pregnant women and infants receiving zidovudine. The pharmacokinetics of HIVIG in 12 maternal-infant pairs participating in ACTG 185 have recently been reported (8). The mean $T_{1/2}$ and CL in these pregnant women were 15 days and 4.1 ml/kg/day, respectively, after the first infusion and 32 days and 4.3 ml/kg/day, respectively, after the third infusion of HIVIG, which was administered at a dosage of 200 mg/kg every 28 days. In the newborns, transplacental passage of anti-p24 antibody was found; the HIVIG $T_{1/2}$ was 30 days, and CL was 4 ml/kg/day after a single 200 mg/kg dose. The second study under way is ACTG 273, a multicenter, randomized, dose-ranging evaluation of HIVIG for slowing the progression of disease in HIV-infected children.

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