Toxicity, Biological Activity, and Pharmacokinetics of TXU (Anti-CD7)-Pokeweed Antiviral Protein in Chimpanzees and Adult Patients Infected with Human Immunodeficiency Virus

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Accepted for publication August 27, 1999 This paper is available online at http://www.jpet.org

ABSTRACT
The purpose of the present study was to evaluate the toxicity and pharmacokinetics of TXU (anti-CD7)-pokeweed antiviral protein (PAP) in human immunodeficiency virus (HIV)-infected chimpanzees and adult patients. At a total dose of 100 µg/kg, TXU-PAP did not cause severe (grade ≥ 3) toxicity in any of the four HIV type 1 (HIV-1)-infected or two healthy chimpanzees. The only side effects were a transient elevation of the liver enzyme alanine aminotransferase between days 2 and 14 without a concomitant rise in total bilirubin levels and a decrease in the serum albumin levels between days 1 and 5 without any concomitant weight gain or peripheral edema. TXU-PAP showed favorable pharmacokinetics in chimpanzees with a plasma elimination half-life of 5.1 to 12.0 h and a systemic clearance of 5.8 to 15.1 ml/h/kg. At 2 months after initiation of TXU-PAP infusions, the HIV-1 burden was reduced to be-
was to evaluate the toxicity and pharmacokinetics of TXU-PAP in HIV-infected chimpanzees and adult patients.

Materials and Methods

Animals. Six adolescent Pan troglodytes chimpanzees were treated according to a contract services agreement at the Coulston Foundation (Alamogordo, NM) or Southwest Foundation (San Antonio, TX). Chimpanzees were fed PMI Fiber-Plus Chimpanzee Diet 5049 or 5037 Jumbo. A veterinarian or designated technician observed each animal daily for signs of illness or distress. The selected daily TXU-PAP dose was administered i.v. over 1 h. Animals were sedated with ketamine or other appropriate anesthetic agents to obtain body weights, physical examination parameters, and collect blood samples. Animals were incubated and maintained on inhalant anesthesia (isoflurane) for the duration of the infusion. Blood samples from all chimpanzees were collected before each infusion and sent for determination of a complete blood cell count with differential and platelets, serum albumin, liver enzyme levels, coagulation parameters, blood urea nitrogen, serum creatinine, and serum electrolytes. Branched-chain DNA (Chiron HIV-1 Quantiplex version 2.0) signal amplification method was used to detect and quantify the HIV-1 viral load, as measured by HIV-1 RNA copies/ml plasma, in peripheral blood of chimpanzees at the indicated time points. Vital signs (including heart rate, respiratory rate, systolic blood pressure, and diastolic blood pressure), as well as overall well-being, were monitored at least twice daily. Body weight was determined daily. Clinical and laboratory data were analyzed and tabulated using the National Cancer Institute toxicity grading system. The chimpanzees were not euthanized after completion of the study.

Patients. Nine HIV-1-infected patients (three African-American women, one African-American man, one Hispanic woman, and four Hispanic men; age range, 27–57 years, median age, 42 years) who were diagnosed with HIV disease and had detectable HIV burden despite antiretroviral therapy were enrolled in this clinical pharmacokinetics study of TXU-PAP between July 14, 1998, and August 22, 1998. These patients were initially diagnosed between 1991 and 1997 (i.e., 1–7 years before study entry), and for all of them, previous treatment programs using combinations of nucleoside inhibitors, protease inhibitors, and/or non-nucleoside inhibitors had failed. To be eligible for this study, patients had to 1) be older than 13 years; 2) have HIV-1 infection as documented with a licensed enzyme-linked immunosorbent assay (ELISA) kit and confirmed by polymerase chain reaction (PCR) detection of HIV-1 RNA in the plasma within 30 days before study entry; 3) have received and failed prior therapy with available licensed anti-HIV drugs; 4) have a CD4+ T cell count of >300/μl within 30 days before study entry; 5) have a Karnofsky performance score of ≥70 within 14 days before study entry; 6) have a plasma hemoglobin level of ≥9.0 g/dl, white blood cell count of ≥3000/μl, absolute neutrophil count of ≥1200/μl, and a platelet count of ≥80,000/μl; 7) have adequate renal function (serum creatinine of ≤1.5 × normal or glomerular filtration rate of >70 ml/min/1.73 m²); adequate liver function (total bilirubin of ≤1.5 × normal and alanine aminotransferase (ALT) ≤5 × normal); adequate cardiac function with ejection fraction ≥50%; adequate pulmonary function (no dyspnea at rest or exercise intolerance and oxygen saturation by pulse oximetry >94% in room air); and 8) be β-human chorionic gonadotropin negative (if female) within 14 days before study entry and practice adequate birth control to prevent pregnancy while receiving the study medication and for 3 months thereafter. Patients with: 1) lymphoma, 2) malignancy (e.g., Kaposi’s sarcoma) requiring systemic therapy, 3) higher than grade II bilateral peripheral neuropathy, 4) history of acute or chronic pancreatitis, 5) active uncontrolled opportunistic infections or unexplained temperature of >38.5°C, 6) severe chronic diarrhea defined as more than three liquid stools/day persisting for ≥15 days within the last 30 days before study entry, 7) uncontrolled diabetes mellitus or other serious medical conditions within 14 days before study entry, or 8) a history of an experimental therapy, interferon or interleukin therapy, or HIV vaccine exposure within 30 days before study entry were not eligible. Patients were treated and followed at the Hughes Institute (St. Paul, MN) and South Florida Bioavailability Clinic (Miami, FL). The clinical protocol was approved by the institutional review boards of the participating Institutions. Informed consent was obtained from all patients or their guardians according to U.S. Department of Health and Human Services Guidelines.

Standard Laboratory Tests in Patients. The CD4+ T cell count and CD56+ natural killer (NK) cell count were determined by flow cytometric immunophenotyping, as described previously (Uckun and Ledbetter, 1988; Uckun et al., 1997). All immunophenotypic analyses were done at the centralized Immunophenotyping and Flow Cytometry Laboratory of the Fairview University Medical Center (St. Paul, MN). Blood samples from all nine patients were also collected before each infusion and sent for determination of a complete blood cell count with differential and platelets, serum albumin, liver enzyme levels, coagulation parameters, blood urea nitrogen, serum creatinine, and serum electrolytes. The determination of the plasma HIV-1 RNA burden by quantitative PCR was performed at the ViroMed Laboratories, Inc. (St. Paul, MN). Reverse transcription-PCR (AMPLICOR HIV-1 MONITOR, Roche Diagnostics) is a target amplification method that simultaneously reverse transcribes and amplifies viral RNA and an internal quantitation standard (QS) RNA and then detects the amplified material in a microwell plate format. The signal strength from the specimen’s RNA is compared with that of the QS, and the concentration of viral RNA is determined. The quality control procedures were as follows: Each batch of specimens tested included one high positive and one low positive control as well as a negative control. The expected range for each of the positive controls was provided with each kit Data Card. If positive controls were out of the published Data Card range, the laboratory supervisor or designee was called on to determine whether the run was valid. A QS was added to each specimen, and two wells containing QS only were included as an assay control. The QS is a synthetic RNA molecule with primer sites that are identical with the HIV target and a unique probe sequence specific for the QS molecule. A known number of QS copies were added to the specimen early in the processing stage such that the QS and the specimen RNA undergo the same reverse transcription, amplification, hybridization, and detection steps. HIV-1 RNA levels were determined by comparing the absorbance of the specimen with that of the QS. The QS controls for any inhibitory influences present in the patient specimen. If either the QS or HIV-1 values were outside of expected limits, the assay was repeated. All raw data and calculated data were verified by an individual who did not perform the test or calculations; the proofed results were entered into the laboratory information management system. Results entered into the laboratory information management system were subsequently reviewed by one of the following before their release: Supervisor of Molecular Biology, Director of Clinical Virology and Molecular Biology, Scientific Director, President of ViroMed Laboratories, Inc.

Dosage and Drug Administration. The procedures used for the large-scale production and purification of TXU-PAP have been previously described in detail (Myers et al., 1997). The composition and physicochemical properties of TXU-PAP were previously reported (Myers et al., 1997). The endotoxin level of TXU-PAP was determined by the limulus amebocyte lysate assay. The endotoxin levels of three lots of TXU-PAP that have been produced were <0.24 EU/mg, <0.59 EU/mg, and <0.85 EU/mg (material used in the present study). The specification for TXU-PAP final product is ≤3 EU/mg. The maximum human dose (M) administered in a single 1-h period was 0.005 mg/kg. Therefore, the acceptable endotoxin limit was K/M (where K = 5.0 EU/kg), which is 5.0/0.005 EU/mg = 1000 EU/mg. Thus, the endotoxin level of the TXU-PAP patient lot was >1000-fold lower than the acceptable limit. The murine DNA contamination was <171 pg DNA/μl (<342 pg DNA/mg). The TXU-PAP preparation contained <13% free TXU monoclonal antibody and <1% free...
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PAP, as determined by immunoblotting using anti-PAP and anti-mouse IgG antibodies. TXU-PAP preparation was not pyrogenic in rabbits and was found to be sterile when tested for bacterial and fungal contamination with the direct inoculation method at Microbiological Associates, Inc. (Rockville, MD). The sterility testing was performed in compliance with the U.S. Food and Drug Administration Good Laboratory Practice regulations (21 CFR 58) and Good Manufacturing Practices Regulations, Title 21 CFR 211 and 610. The purpose of the sterility test was to detect the presence of one or more species of bacterial and fungal contaminants in the test article. The direct inoculation method meets or exceeds USP 23 and/or 21 CFR610 requirements. TXU-PAP did not cause turbidity in the fluid thiglycollate, soybean-casein digest, and peptone yeast glucose broth. No growth was observed on the Sabouraud-dextrose agar slants. The bacterial positive controls did cause turbidity in all broths while growth was observed in the fungal positive control tubes. The uninoculated and the inoculated negative control broth tubes and the agar slants remained clear. The general safety test in mice and guinea pigs was satisfactory. TXU-PAP immunotoxin was formulated as a sterile solution in 150 mM sodium chloride and 40 mM sodium phosphate buffer, pH 7.5, and was stable over 48 months at 4°C as determined by Western blotting. The same TXU-PAP lot was used in chimpanzees and human subjects.

For i.v. administration, TXU (anti-CD7)-PAP was diluted in 100 ml of normal saline. In the preclinical chimpanzee study, four HIV-1-infected chimpanzees were treated with daily infusions of 10 μg/kg/day TXU-PAP i.v. for 10 consecutive days and two healthy chimpanzees were treated with 20 μg/kg/day TXU-PAP i.v. for 5 consecutive days. In the clinical study, nine patients were treated with a single 1-h i.v. infusion of 5 μg/kg TXU-PAP administered 15 to 20 min after premedication (in patients only) with recommended doses of diphenhydramine (1 mg/kg Benadryl) and acetaminophen (650 mg Tylenol) to prevent potential allergic or febrile reactions to this biological agent.

**Pretreatment and Follow-Up Evaluation of Patients.** Medical histories, physical examinations, and laboratory studies were performed before enrollment and throughout therapy to monitor the toxic effects of TXU-PAP. Laboratory studies included a complete blood cell count with differential, serum electrolytes, blood urea nitrogen, creatine, liver function tests (bilirubin, ALT, aspartate aminotransferase, alkaline phosphatase), urine analysis, chest radiography, pulse oximetry, electrocardiography, cardiac pool scan or echocardiography, and a pulmonary function test. Chest radiographs and echocardiograms were repeated on day 14 and whenever clinically indicated. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria. The clinical and laboratory evaluations of patients for toxicity were performed daily on days 1 through 7 and then weekly until day 91 (i.e., days 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, and 91).

**Pharmacokinetic Studies and Pharmacokinetic Modeling.** In the preclinical study, blood samples were collected from the chimpanzees before infusion and at 15 min, 30 min, 1 h, 12 h, and 24 h after completion of the first infusion on day 1 and last infusion on day 10. In addition, preinfusion and 1-h postinfusion blood samples were collected on days 2 through 9. In the clinical study, blood samples were obtained from the HIV-1-infected patients before and at 1, 2, 4, 9, 12, 18, and 24 h after the 1-h TXU-PAP infusion. The concentration of the intact TXU-PAP immunon conjugate in the plasma samples from chimpanzees and patients was determined by a quantitative “double sandwich” solid-phase ELISA detection system, as described in detail (Waurzyniak et al., 1997). The intact TXU-PAP concentrations in the plasma samples were determined from standard curves that were generated by linear regression analysis using varying amounts of purified TXU-PAP standard. The lower limit of detection for this assay is 1 ng/ml.

PK modeling and PK parameter estimations were carried out using commercially available PK software (WinNonlin Program, Standard Version 2.1; Pharsight Corporation, Mountain View, CA). An appropriate PK model was chosen on the basis of the lowest sum of weighted squared residuals, lowest Schwartz Criterion (SC), lowest Akaike’s Information Criterion (AIC) value, lowest standard errors of the fitted parameters, and dispersion of the residuals (Chen et al., 1999; Chen and Uckun, 1999). The elimination half-life was estimated by linear regression analysis of the terminal phase of the plasma concentration-time curve. The area under the concentration-time curve (AUC) was calculated by the trapezoidal rule between the first (0 h) and last sampling time plus C/k, where C is the concentration at the last sampling time and k is the elimination rate constant. Systemic clearance (CL) was determined by dividing the dose by AUC. The mean residence time (MRT) was calculated as the ratio of area under the first moment curve over AUC. For the day 1, PK data of chimpanzee C-291 as well as C-294, the AIC (Akaike Information Criteria, a measure of goodness fit; 3.1) in the two-compartment model was significantly lower than the AIC (22.8) in the one-compartment model. Furthermore, the coefficient of variance (CV) for all parameters after fitting to a two-compartment was <10%, whereas the CV for all parameters was >10% after fitting to a one-compartment model. Therefore, the two-compartment model was determined as the best fit for the day 1 PK data for the chimpanzees. For the day 10 PK data, the AICs for fitting the data to two-compartment versus one-compartment models were similar (32.2 versus 29.6); the CV for all parameters after fitting to a one-compartment model was <15%, whereas the CV for all parameters after fitting to a two-compartment model was >51.7%. Therefore, the one-compartment model was determined as the best fit for the day 10 PK data. The CV for all parameters was <21% (range, 2.2–20.7%), indicating that the models we chose are very appropriate to describe the concentration-time data. The CV for elimination half-life was <15%.

**Anti-HIV Activity of Plasma Samples from TXU-PAP-Treated Adult Patients.** We examined the ability of the 1-h postinfusion samples (1:2 to 1:100 dilution) from four HIV-1-infected patients to inhibit the replication of the HIV-1 strain HTLV-III in normal PBMCs, as described previously for cynomolgus monkey plasma samples (Uckun et al., 1998). The concentration of TXU-PAP needed to inhibit viral replication by 50% was based on p24 production assays (IC50 [p24]). Percent viral inhibition was calculated by comparing the p24 values from the test plasma-treated infected cells with p24 values from untreated infected cells (i.e., virus controls; Uckun et al., 1998; Sudbeck et al., 1998).

**Results**

**Pharmacokinetics, Toxicity, and Biological Activity of TXU-PAP in Chimpanzees.** Four HIV-1-infected chimpanzees (C-291, C-294, C-881, and C-1150) were treated with 10 μg/kg/day TXU-PAP i.v. for 10 days. The therapy was very well tolerated by three of the chimpanzees without any clinical signs of toxicity. One chimpanzee (C-881, Table 1), who did not tolerate the anesthesia well, showed signs of moderate-severe dehydration during days 11 to 21 (weight loss, hypoactivity, increased serum sodium levels, increased levels of blood urea nitrogen, increased heart rate), which was attributed to the side effects of anesthesia, but then fully recovered. All four chimpanzees showed a transient grade I to III elevation of the liver enzyme ALT between days 2 and 14 without a concomitant rise in total bilirubin levels (Table 1). The serum albumin levels decreased between days 1 and 5 without any concomitant weight gain or peripheral edema. This hypoalbuminemia, which we attributed to a very mild presumed grade I vascular leak syndrome (VLS), did not resolve for 2 to 3 weeks (Table 1).

Two HIV-1-negative chimpanzees (C-138 and C-144) were treated with 20 μg/kg/day TXU-PAP i.v. for 5 days. Both
with a large amount of gas was evacuated by slow suction via a gastric tube. Dehydration was also noted.

reepithelialization was observed on toes III, IV, and V.

abrasion, circular with loss of epidermis on the right ventral mandibular branch was noted.

the three areas indicated above showed increased loss of epidermis and inflamed dermis (2.5–3.0-cm diameter along ventral edge of right mandible with increasing

and mild mucopurulent secretions were found: right side of face over ventral edge of mandible, right thorax over the last rib, and central part of the left pinna. On day 15,

was present. A pinpoint area with mild mucous exudation along the ventral edge of the last rib on the right side was also found. On day 14, three areas with epidermal loss

average (mean

Concomitant hypoalbuminemia but no weight gain.

mild grade I VLS as evidenced by a periorbital edema with

chimpanzees showed a transient grade I/II elevation of ALT between days 2 and 5. These chimpanzees also developed a mild grade I VLS as evidenced by a periorbital edema with concomitant hypoalbuminemia but no weight gain.

The pharmacokinetics of TXU-PAP was examined in two of the four HIV-1-infected chimpanzees (C-291 and C-294). The average (mean ± S.E.) trough plasma TXU-PAP levels for days 2 to 10 were 34 ± 5 ng/ml in C-291 and 39 ± 7 ng/ml in C-294. The average peak plasma TXU-PAP levels 1 h postinfusion for days 1 to 10 were 250 ± 34 ng/ml in C-291 and 274 ± 42 ng/ml in C-294. The plasma concentration-time curves for days 1 and 10, which are depicted in Fig. 1, illustrate that TXU-PAP showed a similar PK behavior in the two chimpanzees. A two-compartment PK model was used as the best-fit model to analyze the day 1 plasma concentration-time datasets (Fig. 1A), and a single-compartment PK model was used as the best-fit model to analyze the day 10 plasma concentration-time datasets (Fig. 1B). The reasons for these differences in PK behavior between day 1 and day 10 are unknown. Analytical, sampling, and estimation errors may significantly affect the compartment model chosen. Because analytical and estimation errors were within 20%, we believe that sampling time and frequency may have significantly affected the compartmental model chosen. For example, there was a big sampling gap between 1-h-post and 12-h-post samples, which may significantly affect the model chosen. Alternatively, free antibody released from the immunotoxin may accumulate and compete with binding of the immunotoxin to CD7-positive cells in the extravascular system, leading to the apparent change in PK.
TXU-PAP showed slow elimination in C-291 as well as C-294 with day 1 elimination half-lives of 12.0 and 7.8 h, respectively. The day 10 elimination half-lives were 7.6 h in C-291 and 5.1 h in C-294. These values are similar to the elimination half-lives of 8.1 to 8.5 h observed in TXU-PAP-treated cynomolgus monkeys (Waurzyniak et al., 1997). The estimated volume of distribution at steady state on day 10 (74 ml/kg in C-291 and 62 ml/kg in C-294) was slightly larger than the physiological plasma volume of 40 ml/kg, likely due to the uptake of TXU-PAP by circulating CD7 antigen-positive T cells and monocytes. The estimated day 1 AUC values were 664 ng · h/ml for C-291 and 1711 ng · h/ml for C-294. The estimated day 10 AUC values were 1473 ng · h/ml for C-291 and 1174 ng · h/ml for C-294.

All four HIV-1-infected chimpanzees had PCR evidence of HIV-1 RNA in their plasma before TXU-PAP therapy. At 2 months after initiation of the TXU-PAP infusions, the HIV-1 burden was reduced to below detection levels in three of four chimpanzees and no time course information is available.

**Pharmacokinetics, Toxicity, and Biological Activity of TXU-PAP in HIV-1-Infected Adult Patients.** We examined the pharmacokinetics of TXU-PAP in nine HIV-1-infected adult patients. A one-compartment PK model was used to analyze the plasma concentration-time curves. TXU-PAP showed a slow elimination with a mean ± S.E. plasma elimination half-life of 12.4 ± 1.4 h, an MRT ± S.E. of 17.9 ± 2.0 h, and a mean ± S.E. systemic clearance of 2.7 ± 0.7 ml/h/kg. The mean value for the AUC was 3059 ± 721 ng · h/ml (Table 2). The pharmacokinetic parameters obtained from “pooled data modeling” were very similar to these mean values: T1/2 = 14.2 h; MRT = 20.4 h; clearance = 1.4 ml/h/kg; AUC = 3516 ng · h/ml. The composite plasma concentration-time curve for all patients is depicted in Fig. 2. All nine patients were evaluated for toxicity and developed no significant adverse reactions to TXU-PAP. In particular, no patient developed VLS, allergic reactions, or myalgias.

We examined the in vitro anti-HIV activities of 1-h postinfusion plasma samples from four patients treated with TXU-PAP. The measured TXU-PAP concentrations in these 1-h postinfusion samples were 114 ng/ml (UPN1), 282 ng/ml (UPN2), 109 ng/ml (UPN3), and 118 ng/ml (UPN4), respectively. As shown in Fig. 3, these plasma samples showed potent antiviral activity against HTLVIIIB during a 10-day continuous exposure and inhibited its replication in normal PBMCs even at a 1:100 dilution.

Six of the nine patients agreed to repeated blood sampling for determination of their HIV burden and absolute numbers of circulating CD4+ T and NK cells. As shown in Table 3, treatment with TXU-PAP at this very low dose level, which does not provide sustained therapeutic levels, was capable of reducing the viral burden in all six patients. In five (UPN 1, 2, 3, 4, and 8) of the six patients, we also examined the plasma samples for the presence of HIV-1 p24 antigen. TXU-PAP treatment resulted in reduction of plasma p24 levels of UPN4 by >50%; The plasma p24 levels were 16.0 pg/ml before therapy, 6.0 pg/ml at 4 weeks, 6.3 pg/ml at 5 weeks, 9.1 pg/ml at 6 weeks, and 8.1 pg/ml at 7 weeks. However, at 8

**TABLE 2**

Pharmacokinetic parameter values in HIV-1-infected adult patients

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>Unique Patient Number</th>
<th>Mean</th>
<th>S.E.</th>
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<tr>
<td>Vss (ml/kg)</td>
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<tr>
<td>Cmax (ng/ml)</td>
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<td>149</td>
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<td>Elimination half-life (h)</td>
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<td>12.4</td>
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<td>MRT (h)</td>
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<td>14.4</td>
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<td>Clearance (ml/h/kg)</td>
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<td>0.8</td>
</tr>
<tr>
<td>AUC (ng × h/ml)</td>
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<td>7183</td>
<td>3059</td>
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weeks, the plasma p24 antigen levels increased to 22.7 pg/ml, concomitant with a significant increase in HIV-1 RNA load. Importantly, we observed a significant increase in circulating NK cell numbers in all six patients.

**Discussion**

We evaluated the toxicity, pharmacokinetics, and clinical potential of the TXU (anti-CD7)-PAP immunoconjugate as a new biotherapeutic anti-HIV agent in HIV-1-infected chimpanzees and adult patients. TXU-PAP showed favorable pharmacokinetics in both chimpanzees and adult patients with relatively long elimination half-lives and was very well tolerated at the applied dose levels without any clinical signs of significant toxicity in chimpanzees or adult patients. At 2 months after initiation of the daily $10^3$ TXU-PAP infusions at a 10 μg/kg/day dose level, the HIV-1 burden was reduced to below detection levels in three of the four chimpanzees. In the remaining chimpanzee, the HIV burden became unde-
tectable at 2 weeks but returned to the pretreatment levels by 2 months. We examined the in vitro anti-HIV activities of 1-h postinfusion plasma samples from four patients treated with a single 5 µg/kg dose of TXU-PAP. These plasma samples showed potent anti-HIV activity and inhibited HIV replication in normal PBMCs even at a 1:100 dilution. Furthermore, treatment with TXU-PAP at this very low dose level was capable of reducing the viral burden and increasing circulating NK cell numbers in six of six patients evaluated.

On the basis of its anti-HIV activity in preclinical models, favorable pharmacokinetics, and relative lack of toxicity, we hypothesize that the incorporation of this immunomodulating into clinical treatment protocols may improve the prognosis of HIV-infected patients. A phase I dose-escalation study is now under way to determine the maximum tolerated dose of TXU-PAP when administered as a single infusion per day for 10 consecutive days.

TXU-PAP immunomodulating delivers the broad-spectrum antiviral activity of PAP to CD7 antigen carrying T cells and monococytes (Zarling et al., 1990; Uckun et al., 1998). PAP is a site-specific RNA N-glycosidase that catalytically removes a single adenine base from a highly conserved loop of the large rRNA species in eukaryotic (28S RNA) and prokaryotic (23S rRNA) ribosomes (Monzingo et al., 1993; Hudak et al., 1999). This depurination of the SR loop results in irreversible inhibition of protein synthesis at the translocation step (Ussery et al., 1977; Aron and Irvin, 1980; Kurinov et al., 1999) by impairing both the elongation factor-1-dependent binding of aminoacyl-tRNA and the GTP-dependent binding of elongation factor-2 to the affected ribosome. Our initial studies had suggested that the anti-HIV activity of PAP can be attributed to its ability to reduce viral protein synthesis in HIV-infected cells (Zarling et al., 1990; Uckun et al., 1998). PAP has also been shown to inhibit ribosomal frameshifting and retrotransposition, a molecular mechanism used by many RNA viruses, including HIV-1, to produce Gag-Pol fusion proteins (Tumer et al., 1998). More recent studies indicate that the potent antiviral activity of PAP may at least in part be due to the unique ability of PAP to extensively depurate viral RNA, including HIV-1 RNA (Rajamohan et al., 1999). The emergence of resistance to the antiretroviral agents continues to be a major obstacle to an effective treatment of HIV-infected patients (Carpenter et al., 1998). Because the molecular mechanism of action of TXU-PAP is different from those of the currently available anti-HIV agents, we postulate that the combination of TXU-PAP with other anti-HIV drugs may enhance their anti-HIV activity and reduce the likelihood for the emergence of drug-resistant HIV strains.

References


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