Reduction of soluble CD163, substance P, programmed death 1 and inflammatory markers: phase 1B trial of aprepitant in HIV-1-infected adults

Pablo Tebas[a], Sergei Spitsin[d], Jeffrey S. Barrett[b,d], Florin Tuluc[b,d], Okan Elci[e], James J. Korelitz[e], Wayne Wagner[a], Angela Winters[d], Deborah Kim[a], Renae Catalano[e], Dwight L. Evans[c] and Steven D. Douglas[b,d]

Objective: We evaluated safety, antiviral, immunomodulatory and anti-inflammatory properties of aprepitant – a neurokinin 1 receptor antagonist.

Design: Phase IB randomized, placebo-controlled, double-blinded study.

Methods: Eighteen patients were randomized (nine to aprepitant and nine to placebo). The patients received once-daily treatment (375 mg aprepitant or placebo by oral administration) for 2 weeks and were followed off drug for 4 weeks.

Results: There were no significant changes in the plasma viremia or $CD4^+$ T cells during the dosing period. Aprepitant treatment was associated with significant decreases of median within patient change in percentages of $CD4^+$ T cells expressing programmed death 1 (−4.8%; $P = 0.04$), plasma substance P (−34.0 pg/ml; $P = 0.05$) and soluble CD163 (−563 ng/ml; $P = 0.02$), with no significant changes in the placebo arm. Mean peak aprepitant plasma concentration on day 14 was 7.6 ± 3.1 μg/ml. The use of aprepitant was associated with moderate increases in total cholesterol, low-density lipoprotein and high-density lipoprotein (median change = +31 mg/dl, $P = 0.01$; +26 mg/dl, $P = 0.02$; +3 mg/dl, $P = 0.02$, respectively).

Conclusion: Aprepitant was safe and well tolerated. At the dose used in this proof-of-concept phase IB study, aprepitant did not show a significant antiviral activity. Aprepitant-treated patients had decreased numbers of $CD4^+$ programmed death 1-positive cells and decreased plasma levels of substance P and soluble CD163, suggesting that blockade of the neurokinin 1 receptor pathway has a role in modulating monocyte activation in HIV infection. Prospective studies in virologically-suppressed individuals are warranted to evaluate the immunomodulatory properties of aprepitant. Exposures exceeding those attained in this trial are more likely to elicit clinical benefit.

[a]Department of Medicine, [b]Department of Pediatrics, [c]Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, [d]The Children's Hospital of Philadelphia Research Institute, Philadelphia, Pennsylvania, and [e]Westat, Rockville, Maryland, USA.

Correspondence to Steven D. Douglas, MD, Professor and Associate Chair, Department of Pediatrics, Perelman School of Medicine, Division of Allergy-Immunology, Children's Hospital of Philadelphia, Abramson Building Room 1208A, Philadelphia, PA 19104, USA.

Tel: +1 215 590 3561; e-mail: douglas@email.chop.edu

Introduction

While combined antiretroviral therapy (cART) regimens improve the clinical and virologic outcomes in patients with HIV infection, the goal of complete eradication of HIV from the human body remains elusive [1]. Even in individuals with sustained suppression of viral replication, evidence of chronic inflammation and incomplete restoration of T-cell function persists [2]. This immune dysregulation is associated with increased morbidity and mortality manifesting as an increase in the incidence of cardiovascular events, metabolic disorders and a spectrum of other organ damage. In addition, almost 50% of individuals may develop HIV-associated neurocognitive disorder (HAND), mostly in a mild form [3,4], affecting quality of life, adherence to ART, employment and overall survival. The limitations of the current HIV treatment regimens to curtail this ongoing inflammation justifies continued research in anti-HIV therapeutics of new drugs, with new mechanisms of action targeting residual chronic inflammation and neurocognitive impairment.

Substance P and its preferring receptor, neurokinin 1 receptor (NK1R), are central mediators in the interaction between the immune system and the nervous system [5,6]. Compelling data support the concept that NK1R and the tachykinin, substance P, are important in the pathogenesis of HIV/AIDS [6–9]. HIV-infected men and women have elevated levels of circulating substance P [10,11]. Substance P enhances HIV replication in macrophages and NK1R antagonists inhibit it [8,12–16]. The substance P autocrine loop has a role in regulating cytokine and inflammatory responses [6,8]. The NK1R antagonist aprepitant has unique features which make it an attractive candidate to treat residual inflammation in HIV (for review, see [5,6]). Aprepitant is an US Food and Drug Administration (FDA)-approved antiemetic. Aprepitant crosses the blood–brain barrier [17], and has immunostimulatory and anti-inflammatory properties [5,6,18]. Furthermore, aprepitant and other NK1R antagonists were successfully used to reduce symptoms of depression and insomnia in humans [19–21], and in preclinical studies, NK1R antagonists showed promising results reducing anxiety, addiction and inflammation (reviewed [22]).

We recently demonstrated that substance P increases expression of the CD163 receptor on human monocytes [23]. CD163, the hemoglobin scavenger receptor, is expressed exclusively in cells of the monocyte lineage and is a marker of macrophage polarization [24–26]. A high expression of CD163 was linked to tissue-infiltrating monocytes and was detected on central nervous system macrophages in brains of HIV-positive individuals [27–30]. Pro-inflammatory stimuli lead to shedding of the extracellular portion of CD163 which circulates in the blood as a soluble protein (sCD163). Although the functions of sCD163 are unknown, increased levels of sCD163 occur in several chronic inflammatory diseases including HIV infection [31–33]. Elevated sCD163 in plasma is associated with neurocognitive impairment in HIV infection [34].

There are several immunological pathways which are central in HIV pathogenesis and not completely restored by cART (for review, see [2,35]). One such pathway is the programmed death 1 (PD-1) receptor which suppresses T-cell activation [36,37]. In the context of HIV infection, PD-1 signaling may contribute to accelerated CD4$^+$ T-cell loss, T-cell exhaustion, poor immune recovery and disease progression [38–42]. HIV and other chronic viral infections exploit the PD-1 signaling pathway to alleviate immune constraints on viral replication [43].

The purpose of this clinical trial was to determine the in-vivo safety, antiviral activity and the effect on inflammatory markers by comparing the change in HIV RNA viral load and pro-inflammatory markers after 2 weeks of aprepitant monotherapy in patients with HIV infection not receiving ART. The study follows on a previous pilot study [44] with aprepitant dose escalation from 125–250 mg to 375 mg per day (present study).

Methods

Study design and study procedures

The present study was a phase IB randomized, placebo-controlled, double-blinded study to evaluate the safety, antiviral activity, pharmacokinetics, immune modulatory and anti-inflammatory effects of the NK1R antagonist aprepitant in HIV-infected adults with CD4$^+$ cell count at least 350 cells/μl and plasma viral load at least 2000 copies/ml. Eighteen patients with HIV-1 infection, not receiving ART, were stratified by viral load (< vs. $\geq$20 000 copies/ml) and randomized within each stratum to receive aprepitant 375 mg per day, or placebo for 14 days, and then followed for an additional 28 days. The drug was masked by over-encapsulation. The investigators were blinded to the study assignment of the patients. At the screening visit, previous ART (if any) was assessed, safety laboratory tests were conducted, and all patients underwent testing for HIV-1 co-receptor tropism with the use of a validated phenotypic tropism assay. Patients were also tested for plasma levels of HIV-1 RNA (Amplicor HIV-1 Monitor v1.5; Roche Diagnostics, Indianapolis, Indiana, USA). Participants were then randomized and evaluated at day 0, 3, 7, 10 and 14, while they were receiving aprepitant or placebo, and at day 42, 4 weeks after discontinuing study medication. Additionally, an 8-h pharmacokinetic assessment was performed after the first dose and at day 14.

Participants

Participants were HIV-infected individuals, older than 18 years of age, not receiving ART for at least 16 weeks, with a $CD4^+$ cell count greater than 350 cells/μl, HIV RNA viral load greater than 2000 copies and an R5 tropic virus (Monogram). R5 tropic virus-infected patients were chosen on the basis of the previous observations that aprepitant inhibits HIV-1 infection of macrophages *in vitro* by CCR5-dependent mechanisms [15]. We excluded individuals with a history of cancer and other serious illness, pregnancy, chronic hepatitis B or C infection, individuals with significant laboratory abnormalities, or individuals using steroids or any other immunomodulators or chemotherapy. We also excluded individuals with allergy or hypersensitivity to aprepitant. The study was conducted at AIDS Clinical Trials Unit and the Clinical and Translational Research Center (CTRC) of the Hospital of the University of Pennsylvania in Philadelphia, Pennsylvania, USA. All patients signed a written informed consent. The study was sponsored by the National Institutes of Mental Health, approved by the IRB of the University of Pennsylvania and the US FDA (IND#75 558), and registered in Clinical Trials.gov # NCT01300988.

Pharmacokinetics

A validated, liquid chromatography-tandem mass spectrometry method was utilized for the quantification of aprepitant plasma concentration in HIV-infected patients [45]. Noncompartmental analysis was conducted on aprepitant plasma concentration−time data; peak (C_{max}, T_{max}) and time-averaged exposure metrics (area under the curve) were calculated using WinNonlin version 5.2 (Certara Corporation, Princeton, New Jersey, USA). Pharmacokinetic data were summarized with descriptive statistics and graphical presentation was made using GraphPad Prism version 4 (GraphPad Software, La Jolla, California, USA).

Laboratory assays

Substance P levels in plasma

A modified commercially available antigen competition enzyme immunoassay (EIA) from Cayman Chemical Company (Ann Arbor, Michigan, USA), detection range 4−500 pg/ml, was used for the quantitation of substance P, as previously described [46].

Multiplex cytokine assay

Concentrations of 30 pro-inflammatory cytokines and chemokines including tumor necrosis factor (TNF)α, macrophage inflammatory protein (MIP)-1α, granulocyte-colony stimulating factor (G-CSF), interleukin (IL)-6 and IL-8 in human plasma were measured using human Cytokine/Chemokine Magnetic Bead Panel − Premixed 30 Plex, HCYTMAG-60K-PX30 (Millipore, Billerica, Massachusetts, USA).

Soluble CD163 assay

Soluble CD163 was measured by ELISA assay kit (Trillium Diagnostics, Brewer, Maine, USA) according to manufacturer's recommendations.

Viral tropism

Viral tropism was assessed at days 0 and 14 of the study using the Trofile assay (Monogram Biosciences, San Francisco, California, USA).

Flow cytometry

Blood samples were collected with anticoagulant (EDTA) and stained within 24 h with monoclonal antibodies (including anti-$CD4^+$ and anti-PD-1) conjugated with fluorescent labels purchased from Biolegend (San Diego, California, USA), BD Biosciences (San Jose, California, USA) and Trillium Diagnostics (Bangor, Maine, USA). After red blood cell lysis, cells were fixed and analyzed on an LSR II flow cytometer (BD Biosciences). Compensation for fluorescence spill-over and data analysis was performed using FACSDiva software (BD Biosciences).

Statistical analysis

Descriptive statistical analyses of all data collected were conducted using inferential and graphical exploratory data analytic techniques. Descriptive statistics for continuous variables were summarized by computing the mean, SD, median, minimum value, and maximum value. Changes from baseline to day 14 and from baseline to day 42 were computed for each participant for each continuous variable. Categorical variables were summarized as counts and percentages of total within each treatment group.

Statistical comparisons were made in order to test whether the within-participant changes from baseline to day 14 or day 42 were significantly different than 0, and whether these changes within the aprepitant group were significantly different than the changes within the placebo group. The Wilcoxon signed-rank test was used to test whether the within-participant change for each treatment group was significantly different than 0. The Wilcoxon rank-sum test was used to test whether the within-participant change within the aprepitant group was significantly different than the change in the placebo group. The significance level for two-sided testing was set at 0.05. Stata version 12.1 (StataCorp, College Station, Texas, USA) was used for descriptive statistics, producing graphs and tables, and conducting the statistical tests.

Results

Eighteen participants were enrolled and randomized into two treatment groups, nine in each: aprepitant 375 mg and placebo. All participants completed 14 days of treatment and additional 4 weeks of observation period. Table 1a summarizes the demographic characteristics of the participants (there were no statistically significant differences between the two groups).

Table 1. Patient characteristics and adverse events.

(a) Patient characteristics

	Treatment group	
Characteristic	375 mg	Placebo
Number of patients	9	9
Sex		
Male	6 (67%)	7 (78%)
Female	3 (33%)	2 (22%)
Age (years)		
Median	32.4	41.8
Range	(19–56)	(25–52)
Ethnicity		
Hispanic	1 (11%)	1 (11%)
Non-Hispanic	8 (89%)	8 (89%)
Race		
White	0 (0%)	1 (11%)
Black	9 (100%)	7 (78%)
Other	0 (0%)	1 (11%)
Viral load at screening (copies/ml)		
<20 000	7 (78%)	6 (67%)
≥20 000	2 (22%)	3 (33%)

(b) Adverse events

	Treatment group		
	375 mg (n = 9)	Placebo (n = 9)	Total (N = 18)
Severity [number/(%) of adverse events]	[n (%)]	[n (%)]	[n (%)]
Mild	8 (88.9)	11 (50.0)	19 (61.3)
Moderate	1 (11.1)	11 (50.0)	12 (38.7)
Severe	0 (0.0)	0 (0.0)	0 (0.0)
Body system [number/(%) of patients]	[n (%)]	[n (%)]	[n (%)]
Neurological	1 (11.1)	4 (44.4)	5 (27.8)
Systemic	2 (22.2)	5 (55.5)	7 (38.9)
Gastrointestinal	3 (33.3)	4 (44.4)	7 (38.9)
Respiratory	0 (0.0)	4 (44.4)	4 (22.2)
Skin/dermatological	2 (22.2)	0 (0.0)	2 (11.1)
Musculoskeletal	0 (0.0)	1 (11.1)	1 (5.6)
Urinary	1 (11.1)	0 (0.0)	1 (5.6)
Genitourinary	0 (0.0)	1 (11.1)	1 (5.6)

In the nine patients receiving aprepitant, mean peak aprepitant plasma concentrations were $3.4 \pm 0.8\,\mu g/ml$ on day 1 and $7.6 \pm 3.1\,\mu g/ml$ on day 14, as shown in Fig. 1. The time of peak plasma concentration (T_{max}) was $6.7 \pm 2.0\,h$ on day 1 and $3.8 \pm 3.1\,h$ on day 14.

There were no serious adverse events associated with treatment with 375 mg of aprepitant. Adverse events were mild and self-limited in nature, and were equally frequent in both arms (see Table 1b), which shows the number of adverse events and the number of patients with adverse events in the two study arms.

The HIV RNA viral load and CD4+ cell count for the two study arms during the course of treatment is shown in Fig. 2. There were no statistically or clinically significant changes in HIV RNA viral load over time within either
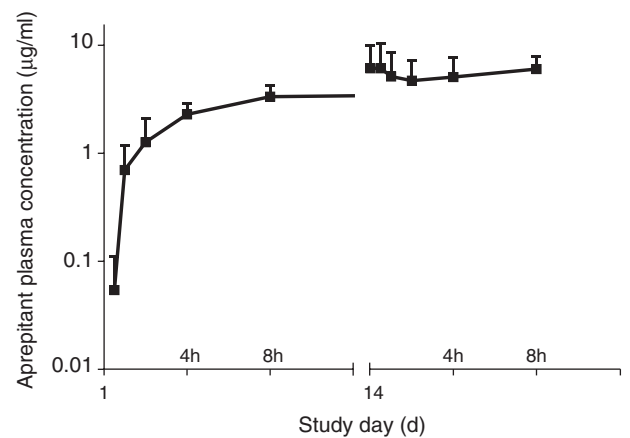

Fig. 1. Plasma levels of aprepitant in patients receiving 375 mg dose per day (dose–exposure relationship). An 8-h pharmacokinetic assessment was performed after the first dose (left) and at day 14 (right). In both cases, blood was drawn at 0.5, 1, 2, 4 and 8 h after 375 mg oral dose of aprepitant. Mean (±SD) aprepitant plasma concentrations in a group of nine patients treated with drug are shown.
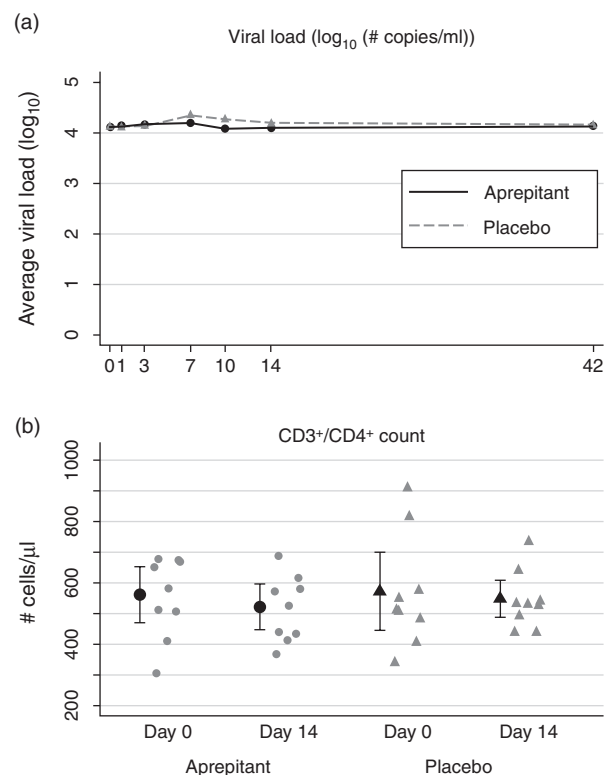

Fig. 2. Viral load and CD4+ T-cell count by treatment arm. Plasma levels of HIV-1 RNA (a) were assessed using Amplicor HIV-1 Monitor v1.5 (Roche Diagnostics) and CD4+ T-cell counts (b) were done using Flow cytometry assay as described in the Methods section. Gray points are the individual CD4+ cell counts for each of the nine aprepitant patients (circles) and nine placebo patients (triangles). Black points are the mean values for the aprepitant group (circles) and the placebo group (triangles). The vertical bars are the widths of the 95% confidence intervals.

the drug or placebo arms, or between the two treatment groups (Fig. 2a). Geometric mean viral load (copies/ml) at baseline for aprepitant and placebo were 13 490 and 13 180, respectively. After 2 weeks of treatment, the viral loads were 12 880 for the aprepitant group and 15 490 for the placebo group. There were no changes in the tropism of the HIV virus in the study participants. All participants' viruses remained R5 tropic, as they were at the initiation of the study. The $CD4^+$ cell counts were stable across all study visits and did not differ significantly by treatment group (Fig. 2b).

There were no statistically or clinically significant differences between the aprepitant and placebo groups in a wide range of laboratory tests monitored during the trial, including hematology (hemoglobin, white blood cell count, hematocrit, absolute neutrophil count, red blood cells, platelets, and mean corpuscular volume); chemistry (serum amylase, potassium, sodium chloride); and liver/kidney functions (direct bilirubin, blood urea nitrogen, aspartate aminotransferase, creatinine, alanine aminotransferase, and albumin) (data not shown).

The use of aprepitant was associated with increases in lipid measurements (Fig. 3). The median within-participant change in total cholesterol in the aprepitant group at the end of treatment (day 14) was +31 mg/dl ($P = 0.01$) (Fig. 3a). Median change in low-density lipoprotein (LDL) cholesterol was +26 mg/dl ($P = 0.02$) and the median change in high-density lipoprotein (HDL) cholesterol was +3 mg/dl ($P = 0.02$) (Fig. 3b and c). These lipid levels generally returned to close to their baseline values by day 42, after treatment was discontinued. No significant changes were observed in the placebo group.

Aprepitant treatment resulted in significant decreases in several pro-inflammatory markers at day 14 that were not seen in the placebo group (Fig. 4). Although we did not see changes in $CD4^+$ T-cell counts, we did observe a median reduction in PD-1 expression by 4.8% ($P = 0.04$) (Fig. 4a). This effect was transient and limited to the treatment phase. PD-1 expression returned to baseline by day 42. No significant changes in PD-1 expression were detected in the placebo group. Plasma substance P levels at day 14 decreased in the aprepitant group (median change = −34 pg/ml, $P = 0.05$), but not in the placebo group (median change = +30 pg/ml, $P = 0.55$) (Fig. 4b). By day 42, however, the values in both the groups were similar to the baseline levels. sCD163 levels decreased in the aprepitant group at day 14 (median change = −563 ng/ml, $P = 0.02$), and remained below baseline at day 42, although no longer statistically significant (median change = −594 ng/ml, $P = 0.09$) (Fig. 4c). No changes were observed in the sCD163 level within the placebo group.

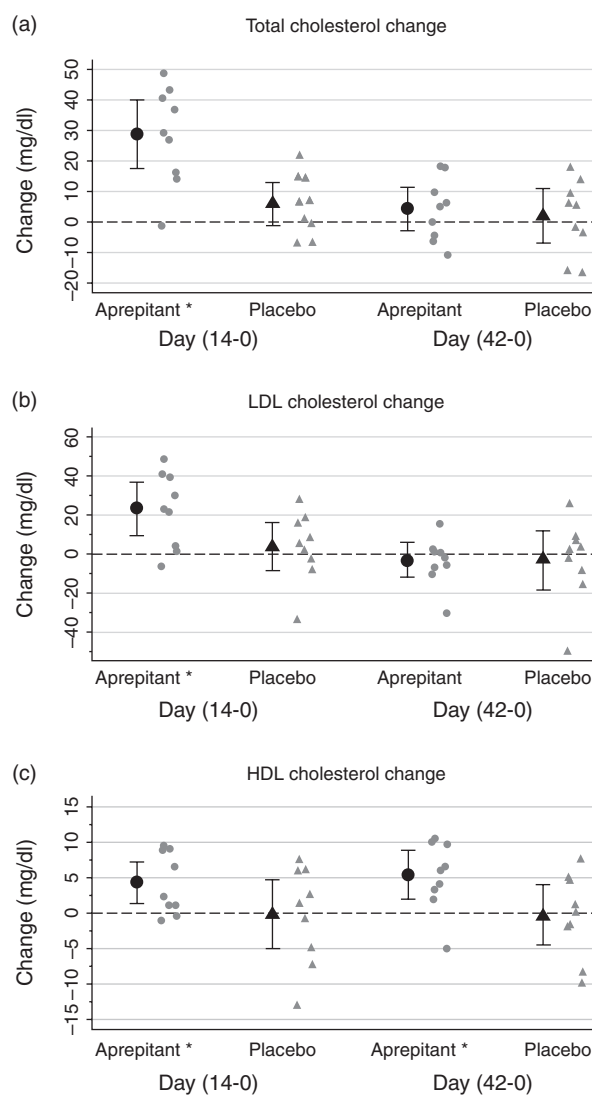Plasma levels of 30 cytokines and chemokines were measured using multiplex assays. Data were obtained for

Fig. 3. Changes in cholesterol levels associated with aprepitant treatment. Total cholesterol, LDL and HDL were measured by Quest Diagnostics (Madison, New Jersey, USA). Results are presented as changes in cholesterol levels in the aprepitant and the placebo-treated groups between day 0 and 14, or day 0 and 42, as indicated. Gray points are the individual changes for each of the nine aprepitant patients (circles) and the nine placebo patients (triangles). Black points are the mean changes for the aprepitant group (circles) and the placebo group (triangles). Vertical bars are the widths of the 95% confidence intervals. Significant changes ($P < 0.05$ using Wilcoxon signed-rank test) are indicated with (*).

26 markers with 4 (IL-3, IL-4, IL-13, and TNFβ) were below the assay range. Although not statistically significant, decreases in the aprepitant group were observed for TNFα, MIP-1α, G-CSF, IL-6, and IL-8 (data not shown). When data from this study were combined with data from the earlier trial that used aprepitant doses of 125 and 250 mg [44], we detected significant within-patient decreases for the combined aprepitant group for all five markers. There were also
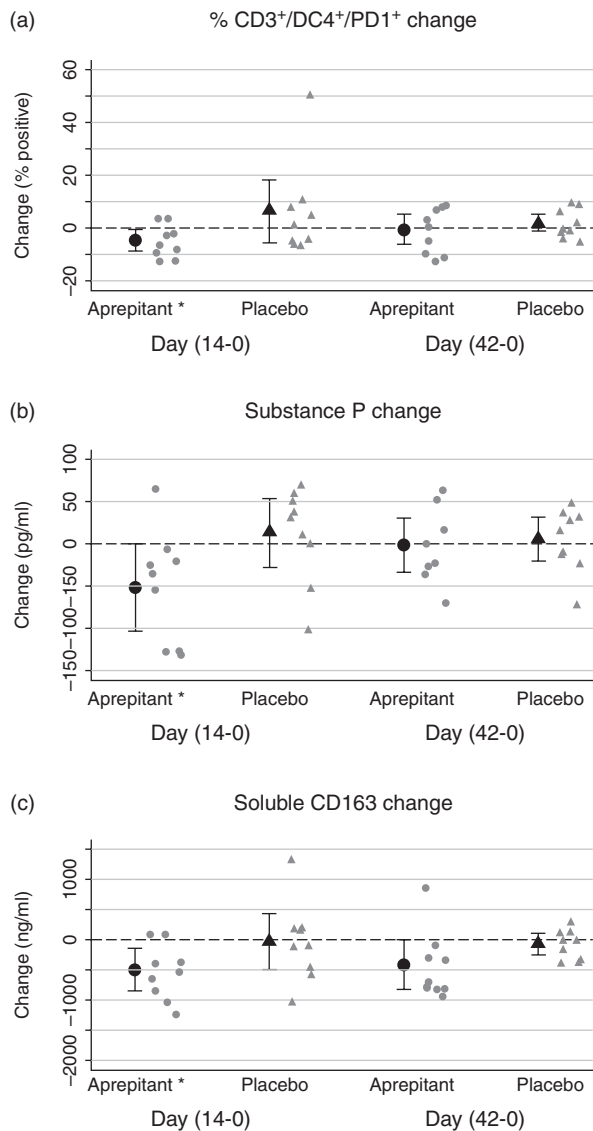
Fig. 4. Changes in pro-inflammatory markers associated with aprepitant treatment. Expression of PD-1 on CD4$^+$ T cells was measured by flow cytometry, and SP and sCD163 levels by ELISA as described in the Methods section. Results are presented as changes in marker levels in the aprepitant and the placebo-treated groups between day 0 and 14, or day 0 and 42, as indicated. Gray points are the individual changes for each of the nine aprepitant patients (circles) and the nine placebo patients (triangles). Black points are the mean changes for the aprepitant group (circles) and the placebo group (triangles). Vertical bars are the widths of the 95% confidence intervals. Significant changes ($P < 0.05$ using Wilcoxon signed-rank test) are indicated with (*).

significant differences between the aprepitant and the placebo groups for TNFα and IL-6 (see Supplemental Digital Content 1, http://links.lww.com/QAD/A671). Changes in plasma levels of the remaining 21 markers are shown in Supplemental Digital Content 2 (http://links.lww.com/QAD/A671). When aprepitant treatment

was discontinued, expression of all markers returned to their baseline level, and no significant changes were detected between day 0 and day 42 for any of the 26 markers analyzed (data not shown).

Discussion

Treatment of HIV viremic patients with daily 375 mg of aprepitant was safe and well tolerated over the 2 weeks of drug administration. The 375 mg per day regimen resulted in plasma concentrations of aprepitant similar to those predicted from the population-based pharmacokinetics model on day 1 [47], but higher than that predicted on day 14 (more than 50% of the observations were above the 90% prediction interval from the simulation model). This is likely related to the observed nonlinearity with dose previously reported [48]. Erratic trough levels suggest that some noncompliant dosing may have contributed to the greater variation in exposures on day 14, but there is also likely to be a saturable absorption effect consistent with previous observations with aprepitant [48]. Despite these higher exposures on day 14, these levels were still below target trough exposures for significant antiviral activity on the basis of data from preclinical, clinical, and competitive surveillance data [47,49]. Despite the increase of dose and plasma levels of aprepitant in the current trial, no increase in frequency of side effects was observed in comparison to the placebo group. This is consistent with the safety profile observed in the previous study that used 125 and 250 mg dose groups [44].

Two weeks duration of aprepitant treatment at a dose of 375 mg showed no clinically (or statistically) significant antiviral effect or increase in CD4$^+$ T cells. These results are consistent with our study that used 125 and 250 mg doses [44]. Previously, we reported antiviral effect of aprepitant and other NK1R antagonists in ex-vivo studies [14–16,49] using HIV infection with both laboratory strains and primary isolates of T cells and macrophages. The observed ED$_{50}$ of aprepitant was 5 μmol/l (2.65 μg/ml) in the ex-vivo studies. We also observed an antiviral effect of aprepitant *in vivo*, in simian immunodeficiency virus (SIV) infection, in a nonhuman primate model [49]. About 1 log$_{10}$ reduction in plasma SIV load was sustained for a period of 1 year. In rhesus macaques receiving 125 mg of aprepitant daily, the mean trough concentration at steady state was 2.66 ± 1.55 μg/ml, with a maximum value of 16–18 μg/ml [47,49].

Despite the increase in aprepitant plasma concentrations in patients receiving 375 mg dose relative to exposure attained during our previous study at lower doses, we think this dose is still too low to observe an antiviral effect based on the target concentrations required to elicit an

antiviral effect from in-vitro and preclinical experiments [49]. In primate studies where an antiviral effect was detected, we observed 2−4-fold higher total plasma concentrations of aprepitant compared to those achieved in the present study [49]. It is also consistent with our modeling results, which suggest that daily dosing of at least 625 mg with exposure enhancement with concomitant dosing of a CYP 3A4 inhibitor (e.g. ritonavir or cobicistat) would yield desirable troughs (based on in-vitro infectivity experiments and the metabolic pathway of aprepitant) of above 2.65 μg/ml total (free + bound) plasma aprepitant concentration for the majority of the virtual patients simulated [47]. Regarding the time course of these effects, primate studies indicated that the antiviral effect of aprepitant was not detected until 1 month after treatment initiation [49]. This is likely explained by an aprepitant mechanism of action that is not due to direct antiviral activity, but rather due to blocking of substance P-driven macrophage polarization and inflammation [23].

During our previous trial, we detected a 5−6% reduction in plasma substance P levels after 2 weeks of treatment with 125 and 250 mg of aprepitant, though changes were not statistically significant [44]. In the current study, we observed a reduction of 7% in plasma substance P levels ($P = 0.05$). We previously reported a 30−40% increase in plasma levels of substance P in HIV-infected individuals [10,11], in comparison to healthy control participants. The decrease of substance P levels in aprepitant-treated patients supports our hypothesis of the autocrine regulation of substance P expression based on our ex-vivo studies with another substance P antagonist CP-96,345 [50].

In a previous trial, a similar reduction was observed in the sCD163 levels (unpublished data). A decrease (10%; $P = 0.18$) was observed in our 125−250 mg study [44] (not shown). A much larger decrease of 20% sCD163 was observed in the current 375 mg study ($P = 0.02$). Plasma levels of several pro-inflammatory cytokines were also decreased in both the 125−250 mg and 375 mg studies. In order to increase the relatively small sample sizes in each study, we combined the data for all the three aprepitant dose groups from our two studies (125, 250, and 375 mg) and the two placebo groups. There was a significant decrease in several cytokines at day 14 in the combined aprepitant group, but not in the placebo group, in this post-hoc analysis. This suggests the anti-inflammatory effects of aprepitant should be evaluated further in a larger sample. Up-regulation of pro-inflammatory markers including TNFα, IL-6, IL-8, and CD163 by substance P, and down-regulation as a result of aprepitant treatment are consistent with the previous observations [6,23,51,52]. The mechanisms of the aprepitant effect may involve inhibition of nuclear factor (NF)-κB activation by substance P (reviewed [5,6]). Furthermore, decrease of

substance P levels by aprepitant contributes to its anti-inflammatory effect.

There are no previous studies on the direct effect of substance P on PD-1 expression. In this study, we demonstrated reduction of PD-1 expression in CD4$^+$ cells in aprepitant-treated patients.

We observed increases in plasma lipids associated with the use of aprepitant. There are no available data on the direct effect of either substance P or aprepitant on cholesterol metabolism. Patients with advanced HIV infection have low HDL-cholesterol levels and other lipid abnormalities including hypertriglyceridemia and increased levels of small LDL particles (reviewed [53]) that may be directly or indirectly related to the high circulating levels of substance P in HIV-infected individuals [10,11]. In addition, substance P may affect lipid metabolism. Increased substance P levels are associated with the development of obesity, chronic inflammation, and type 2 diabetes mellitus [54] and NK1R−/− mice are more resistant to weight gain than normal controls [55].

In conclusion, at the 375 mg dose used in this exploratory phase IB study, aprepitant was safe and showed significant biological activity, but without significant antiviral activity. Aprepitant treatment was associated with decreased PD-1 expression on CD4$^+$ T cells, and decreased plasma levels of substance P and sCD163. Further, we detected decrease in plasma levels of several pro-inflammatory cytokines including IL-6 and TNFα, when samples from the combined 125−250−375 mg studies were analyzed. In chronic HIV infection, increased levels of these markers are associated with poor prognosis. Pharmacokinetic studies showed that aprepitant concentrations could reach as high as 9.2 ± 3.6 μg/ml without enzymatic induction of the aprepitant metabolism. Further studies to evaluate the immunomodulatory and anti-inflammatory effects of aprepitant with longer treatment (4 weeks) and co-administration with ritonavir to boost aprepitant plasma concentrations in virologically suppressed patients on cART are ongoing (Clinical Trials.gov # NCT02154360).

Acknowledgements

graciously recruited and followed the subjects for this study. We thank Nancy Tustin and Richard Tustin III for technical support.

Conflicts of interest

There are no conflicts of interest.

References

1. Eisele E, Siliciano RF. **Redefining the viral reservoirs that prevent HIV-1 eradication.** *Immunity* 2012; **37**:377–388.
2. Lederman MM, Funderburg NT, Sekaly RP, Klatt NR, Hunt PW. **Residual immune dysregulation syndrome in treated HIV infection.** *Adv Immunol* 2013; **119**:51–83.
3. Heaton RK, Clifford DB, Franklin DR Jr, Woods SP, Ake C, Vaida F, et al. **HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study.** *Neurology* 2010; **75**:2087–2096.
4. McGuire JL, Barrett JS, Vezina HE, Spitsin S, Douglas SD. **Adjuvant therapies for HIV-associated neurocognitive disorders.** *Ann Clin Transl Neurol* 2014; **1**:938–952.
5. Douglas SD, Leeman SE. **Neurokinin-1 receptor: functional significance in the immune system in reference to selected infections and inflammation.** *Ann N Y Acad Sci* 2011; **1217**:83–95.
6. Tuluc F, Lai JP, Kilpatrick LE, Evans DL, Douglas SD. **Neurokinin 1 receptor isoforms and the control of innate immunity.** *Trends Immunol* 2009; **30**:271–276.
7. Douglas SD, Lai JP, Tuluc F, Schwartz L, Kilpatrick LE. **Neurokinin-1 receptor expression and function in human macrophages and brain: perspective on the role in HIV neuropathogenesis.** *Ann N Y Acad Sci* 2008; **1144**:90–96.
8. Ho WZ, Douglas SD. **Substance P and neurokinin-1 receptor modulation of HIV.** *J Neuroimmunol* 2004; **157**:48–55.
9. Li Y, Douglas SD, Song L, Sun S, Ho WZ. **Substance P enhances HIV-1 replication in latently infected human immune cells.** *J Neuroimmunol* 2001; **121**:67–75.
10. Douglas SD, Ho WZ, Gettes DR, Cnaan A, Zhao H, Leserman J, et al. **Elevated substance P levels in HIV-infected men.** *AIDS* 2001; **15**:2043–2045.
11. Douglas SD, Cnaan A, Lynch KG, Benton T, Zhao H, Gettes DR, et al. **Elevated substance P levels in HIV-infected women in comparison to HIV-negative women.** *AIDS Res Hum Retroviruses* 2008; **24**:375–378.
12. Bost KL. **Tachykinin-modulated antiviral responses.** *Front Biosci* 2004; **9**:1994–1998.
13. Ho WZ, Cnaan A, Li YH, Zhao H, Lee HR, Song L, et al. **Substance P modulates human immunodeficiency virus replication in human peripheral blood monocyte-derived macrophages.** *AIDS Res Hum Retroviruses* 1996; **12**:195–198.
14. Lai JP, Ho WZ, Zhan GX, Yi Y, Collman RG, Douglas SD. **Substance P antagonist (CP-96,345) inhibits HIV-1 replication in human mononuclear phagocytes.** *Proc Natl Acad Sci U S A* 2001; **98**:3970–3975.
15. Wang X, Douglas SD, Lai JP, Tuluc F, Tebas P, Ho WZ. **Neurokinin-1 receptor antagonist (aprepitant) inhibits drug-resistant HIV-1 infection of macrophages in vitro.** *J Neuroimmune Pharmacol* 2007; **2**:42–48.
16. Manak MM, Moshkoff DA, Nguyen LT, Meshki J, Tebas P, Tuluc F, et al. **Anti-HIV-1 activity of the neurokinin-1 receptor antagonist aprepitant and synergistic interactions with other antiretrovirals.** *AIDS* 2010; **24**:2789–2796.
17. Hargreaves R. **Imaging substance P receptors (NK1) in the living human brain using positron emission tomography.** *J Clin Psychiatry* 2002; **63 (Suppl 11)**:18–24.
18. Monaco-Shawver L, Schwartz L, Tuluc F, Guo CJ, Lai JP, Gunnam SM, et al. **Substance P inhibits natural killer cell cytotoxicity through the neurokinin-1 receptor.** *J Leukoc Biol* 2011; **89**:113–125.
19. Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, et al. **Distinct mechanism for antidepressant activity by blockade of central substance P receptors.** *Science* 1998; **281**:1640–1645.
20. Ratti E, Bettica P, Alexander R, Archer G, Carpenter D, Evoniuk G, et al. **Full central neurokinin-1 receptor blockade is required for efficacy in depression: evidence from orvepitant clinical studies.** *J Psychopharmacol* 2013; **27**:424–434.
21. Ratti E, Carpenter DJ, Zamuner S, Fernandes S, Squassante L, Danker-Hopfe H, et al. **Efficacy of vestipitant, a neurokinin-1 receptor antagonist, in primary insomnia.** *Sleep* 2013; **36**:1823–1830.
22. Steinhoff MS, von Mentzer B, Geppetti P, Pothoulakis C, Bunnett NW. **Tachykinins and their receptors: contributions to physiological control and the mechanisms of disease.** *Physiol Rev* 2014; **94**:265–301.
23. Tuluc F, Meshki J, Spitsin S, Douglas SD. **HIV infection of macrophages is enhanced in the presence of increased expression of CD163 induced by substance P.** *J Leukoc Biol* 2014; **96**:143–150.
24. Graversen JH, Madsen M, Moestrup SK. **CD163: a signal receptor scavenging haptoglobin-hemoglobin complexes from plasma.** *Int J Biochem Cell Biol* 2002; **34**:309–314.
25. Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK, et al. **Identification of the haemoglobin scavenger receptor.** *Nature* 2001; **409**:198–201.
26. Moller HJ, Peterslund NA, Graversen JH, Moestrup SK. **Identification of the hemoglobin scavenger receptor/CD163 as a natural soluble protein in plasma.** *Blood* 2002; **99**:378–380.
27. Fischer-Smith T, Tedaldi EM, Rappaport J. **CD163/CD16 coexpression by circulating monocytes/macrophages in HIV: potential biomarkers for HIV infection and AIDS progression.** *AIDS Res Hum Retroviruses* 2008; **24**:417–421.
28. Kim WK, Alvarez X, Fisher J, Bronfin B, Westmoreland S, McLaurin J, et al. **CD163 identifies perivascular macrophages in normal and viral encephalitic brains and potential precursors to perivascular macrophages in blood.** *Am J Pathol* 2006; **168**:822–834.
29. Soulas C, Conerly C, Kim WK, Burdo TH, Alvarez X, Lackner AA, et al. **Recently infiltrating MAC387(+) monocytes/macrophages a third macrophage population involved in SIV and HIV encephalitic lesion formation.** *Am J Pathol* 2011; **178**:2121–2135.
30. Tippett E, Cheng WJ, Westhorpe C, Cameron PU, Brew BJ, Lewin SR, et al. **Differential expression of CD163 on monocyte subsets in healthy and HIV-1 infected individuals.** *PLoS One* 2011; **6**:e19968.
31. Burdo TH, Lentz MR, Autissier P, Krishnan A, Halpern E, Letendre S, et al. **Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after antiretroviral therapy.** *J Infect Dis* 2011; **204**:154–163.
32. Burdo TH, Lo J, Abbara S, Wei J, DeLelys ME, Preffer F, et al. **Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients.** *J Infect Dis* 2011; **204**:1227–1236.
33. Moller HJ. **Soluble CD163.** *Scand J Clin Lab Invest* 2012; **72**:1–13.
34. Burdo TH, Weiffenbach A, Woods SP, Letendre S, Ellis RJ, Williams KC. **Elevated sCD163 in plasma but not cerebrospinal fluid is a marker of neurocognitive impairment in HIV infection.** *AIDS* 2013; **27**:1387–1395.
35. Hunt PW. **HIV and inflammation: mechanisms and consequences.** *Curr HIV/AIDS Rep* 2012; **9**:139–147.
36. Said EA, Dupuy FP, Trautmann L, Zhang Y, Shi Y, El-Far M, et al. **Programmed death-1-induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection.** *Nat Med* 2010; **16**:452–459.

37. Okazaki T, Honjo T. **The PD-1-PD-L pathway in immunological tolerance.** *Trends Immunol* 2006; **27**:195–201.

38. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. **PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression.** *Nature* 2006; **443**:350–354.

39. Holm M, Pettersen FO, Kvale D. **PD-1 predicts CD4 loss rate in chronic HIV-1 infection better than HIV RNA and CD38 but not in cryopreserved samples.** *Curr HIV Res* 2008; **6**:49–58.

40. Trabattoni D, Saresella M, Biasin M, Boasso A, Piacentini L, Ferrante P, et al. **B7-H1 is up-regulated in HIV infection and is a novel surrogate marker of disease progression.** *Blood* 2003; **101**:2514–2520.

41. Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, et al. **Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction.** *Nat Med* 2006; **12**:1198–1202.

42. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. **Restoring function in exhausted CD8 T cells during chronic viral infection.** *Nature* 2006; **439**:682–687.

43. Freeman GJ, Wherry EJ, Ahmed R, Sharpe AH. **Reinvigorating exhausted HIV-specific T cells via PD-1-PD-1 ligand blockade.** *J Exp Med* 2006; **203**:2223–2227.

44. Tebas P, Tuluc F, Barrett JS, Wagner W, Kim D, Zhao H, et al. **A randomized, placebo controlled, double masked phase IB study evaluating the safety and antiviral activity of aprepitant, a neurokinin-1 receptor antagonist in HIV-1 infected adults.** *PLoS One* 2011; **6**:e24180.

45. Wu D, Paul DJ, Zhao X, Douglas SD, Barrett JS. **A sensitive and rapid liquid chromatography-tandem mass spectrometry method for the quantification of the novel neurokinin-1 receptor antagonist aprepitant in rhesus macaque plasma, and cerebral spinal fluid, and human plasma with application in translational NeuroAIDs research.** *J Pharm Biomed Anal* 2009; **49**:739–745.

46. Campbell DE, Bruckner P, Tustin NB, Tustin R 3rd, Douglas SD. **Novel method for determination of substance P levels in unextracted human plasma by using acidification.** *Clin Vaccine Immunol* 2009; **16**:594–596.

47. Barrett JS, Bajaj G, McGuire J, Wu D, Spitsin S, Moorthy G, et al. **Modeling and simulation approach to support dosing and study design requirements for treating HIV-related neuropsychiatric disease with the NK1-R antagonist aprepitant.** *Curr HIV Res* 2014; **12**:121–131.

48. EMA. EPAR scientific discussion. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000527/WC500026534.pdf (2006).

49. Barrett JS, Moorthy WD, Srivastata G, Barrett P, Spitsin KJ, Tuluc S, et al. **Preclinical activity predicts higher dosing requirements for the NK-1r antagonist aprepitant in HIV-associated neuro-cognitive disorders (HAND): Dispositional and pharmacologic rationale for multimodal therapeutic window.** *Clin Pharmacol Therapeut* 2013; **93 (Suppl 1)**:S17PI-9.

50. Lai JP, Ho WZ, Yang JH, Wang X, Song L, Douglas SD. **A nonpeptide substance P antagonist down-regulates SP mRNA expression in human mononuclear phagocytes.** *J Neuroimmunol* 2002; **128**:101–108.

51. Kepler CK, Markova DZ, Hilibrand AS, Vaccaro AR, Risbud MV, Albert TJ, et al. **Substance P stimulates production of inflammatory cytokines in human disc cells.** *Spine (Phila Pa 1976)* 2013; **38**:E1291–E1299.

52. Spitsin S, Tuluc F, Meshki J, Ping Lai J, Tustin Iii R, Douglas SD. **Analog of somatostatin vapreotide exhibits biological effects in vitro via interaction with neurokinin-1 receptor.** *Neuroimmunomodulation* 2013; **20**:247–255.

53. Giannarelli C, Klein RS, Badimon JJ. **Cardiovascular implications of HIV-induced dyslipidemia.** *Atherosclerosis* 2011; **219**:384–389.

54. Fu J, Liu B, Liu P, Liu L, Li G, Wu B, et al. **Substance P is associated with the development of obesity, chronic inflammation and type 2 diabetes mellitus.** *Exp Clin Endocrinol Diabetes* 2011; **119**:177–181.

55. Karagiannides I, Stavrakis D, Bakirtzi K, Kokkotou E, Pirtskhalava T, Nayeb-Hashemi H, et al. **Substance P (SP)-neurokinin-1 receptor (NK-1R) alters adipose tissue responses to high-fat diet and insulin action.** *Endocrinology* 2011; **152**:2197–2205.