Crack cocaine: effect modifier of RNA viral load and CD4 count in HIV infected African American women

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1. ABSTRACT

This study reports on the role of cocaine as effect modifier of the association of CD4+ cell counts and RNA viral load. HIV-1 seropositive (n=80) and seronegative (n=42) African American women (AAW) crack cocaine smokers were recruited. Increasing cocaine use, based on self-reports and laboratory values, significantly exacerbates the immunopathology of HIV-1 in a dose-response manner, confirmed by a non-linear drop in CD4+ cell number for a given viral load in HIV+ AAW. This report supports a view of deleterious effects due to cocaine use in humans.

2. INTRODUCTION

Presently, plasma virus load and blood CD4+ cell counts are two of the most significant predictive parameters utilized to assess the progression of human immunodeficiency virus (HIV) among infected individuals (1). Complex relationships between CD4+ cell counts and virus load have been consistently used to classify individuals among stages of progression (2), with progression referring in part to the well-documented rise in viral load and the concomitant fall in CD4+ cell counts in the years following HIV infection (2-5). Some report the CD4+ cell decrease is due to increased T cell death due to the HIV infection (6) while others propose lowered T cell production as the cause (7). Others have shown uninfected CD4+ cell depletion, possibly through apoptotic, non-apoptotic, and/or CT8+ CTL (cytotoxic T lymphocyte) -related mechanisms (8-12). There is also some evidence for an inverse relationship between HIV-1-specific CTLs and plasma virus load (13). A negative association of moderate magnitude between RNA viral load and CD4+ cell counts among untreated HIV infected subjects has also been reported (14).

A majority of studies in the literature in this area have focused on whether mean values of CD4+ counts or RNA viral load are different among stratified levels of concomitant variables (15-17). Interestingly, the question of whether the fundamental association between CD4+ cell counts and viral load is affected by drug abuse has not been explored, yet early in the epidemic, it was hypothesized that drug abuse modified disease progression (18,19). One study reported short-term (3-4 months) declines in circulating CD4+ cell numbers among study participants who reported using cocaine during the interval as compared...
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to other study participants who reported no drug use in the same interval (15). However, recent documentations on this subject are controversial. For example, both in vitro and in vivo studies report deleterious effects of drug abuse on the immune system and theorize that drug abuse should promote increased HIV-1-load and HIV-1-related disease progression (20-23). Nevertheless, the relationship between HIV replication, AIDS progression and CD4 counts in human studies have produced incongruous results (24-34). The assessment of statistical associations between a dependent variable such as CD4+ counts and a predictor variable such as RNA viral load are often expressed in the form of multiple regression statistical models which include possible confounding variables such as age, ethnicity, and drugs of abuse. In this form, the confounding variables enter the statistical models on an equal footing with the predictor variable. The intent of such an analysis is to determine whether the effect of RNA viral load on CD4+ counts does or does not change when the confounders are taken into account. There is an indication that cocaine and other drugs of abuse (e.g., alcohol) may not be acting directly, but instead, may be acting as “effect modifiers” on the relationship between viral load and CD4+ counts through indirect effects on the immune system (35). The statistical assessment of effect modifiers casts the concomitant variables in different roles in the multiple regression model as compared to confounder assessment. The statistical question asked is whether the relationship between the dependent variable and the predictor variable is the same across stratified levels of the potential effect modifier. If not, then the concomitant variable “modifies the effect” of the predictor variable. Such analyses are the basis of the common bioassay methods. This report focuses on the role of one of the major drugs of abuse (cocaine) as effect modifier of the association of CD4+ cell counts and RNA viral load.

3. METHODS

3.1. Subject Recruitment

An out-of-treatment “street” population of HIV-1 seropositive and seronegative African American women (AAW) who were crack cocaine smokers were recruited for the study. A purposive snowball sampling technique was utilized as the recruitment strategy and is described in detail elsewhere (36-38). Exclusion criteria included heroin use, combined heroin and cocaine (speedball) use, and present pregnancy. The cases consisted of 80 HIV+ subjects who had observations on all of the relevant variables including current age, time since HIV infection, CD4+ cell count, RNA viral load, and reported days of crack cocaine use in the last 30 days. The control group consisted of 42 HIV-1 seronegative AAW subjects with similar study entry and exclusion criteria, as well as similar demographic information as the cases.

3.2. Virology and Flow Cytometry Methods

Subjects donated two aliquots of blood from an antecubital vein for this study. Fifteen ml were drawn with EDTA as anticoagulant (for plasma virus load) and 10 ml with heparin for cell flow cytometry (39-41). Four ml of heparinized blood was sent overnight to UCLA (Los Angeles, California) flow cytometry studies using standard dual fluorescence flow cytometry methods, and commercial anti-human CD monoclonal antibodies (Becton Dickinson, San Jose, CA) and a FACScan instrument (Becton Dickinson). Absolute CD4+ cell numbers were derived from transformation of the white blood cell count with the differential and the flow analysis data (27,42). Plasma was separated from the EDTA blood by centrifugation at 800g for 10 minutes. The Roche HIV Monitor test was used for virus load determination in plasma (33,41,43). All National Institute of Health and University of Miami human subject requirements were adhered to, including NIH human subjects course attendance by all investigators.

3.3. Measurement of Cocaine Levels

Drug use self-report histories were obtained from all the subjects. (44,45) Urine was analyzed using a drug-screen (EMIT) for cocaine, benzoylecgonine, cocaethylene, benzodiazepine, opiates, tetrahydrocannabinoids and ethanol at the Dade County Medical Examiner’s toxicology laboratory (Ontrack [Roche Diagnostics, Inc., Laval, QB, CN]) (46,47).

3.4. Statistical Methods

Initially, a slope-ratio bioassay relating lnCD4+ counts to RNA viral load as modified by self reports of crack cocaine use or laboratory measured levels of urinary cocaine was performed. Choosing to express CD4+ counts as a function of viral load is predicated on the concept that viremia is sustained by the reinfection and destruction of CD4+ cells (48,49). The slope-ratio assay is based on a simultaneous fitting of a pooled linear regression model relating lnCD4+ as the dependent variable to RNA viral load as the independent variable within stratified levels of reported crack use or urinary cocaine. Often, stratified analyses are reported on the basis of independent regression lines within each of k strata: $Y_{ij} = \beta_0 + \beta_1 X_{ij} + \varepsilon_{ij}$, i=1,2,…,nj and j=1,2,…,k. This formulation is useful for a preliminary investigation of the appropriateness of the proposed model. However, the assay procedure allows each stratum to have a different regression slope but all lines must have a common intercept. The simultaneous fit is accomplished by performing a multiple regression analysis on the model: $Y_{ij} = \beta_0 + \beta_1 X_{ij} + \beta_2 X_{kj} + \ldots + \beta_k X_{ik}; i=1,2,\ldots,N; j=1,2,\ldots,n_j$, where N= n_1 + n_2 + \ldots + n_k, and X_0 = X_k in stratum k and zero elsewhere. Dummy variables can be used to test for homogeneity of intercepts. The covariance matrix of the estimates of the stratum slopes is used to compute the standard errors of the ratios of the stratum slopes using the principles of the propagation of error following the analytical methods found in Finney (50). If the ratio of two slopes is significantly different from one, then the “effect” of the predictor variable is “modified” by the stratifying variable.

Stratum groups for crack usage were defined as “None” if there was no reported crack use in the last month, “Less Than Daily” if the reported crack use was 1-27 days, and “Daily” if the reported use was 28 days or more. Urinary cocaine levels usage groups were stratified by tertiles of the urinary cocaine distribution. Within each
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stratum regression diagnostics (leverage, Cook’s D, and DFBETAS) associated with SAS PROC REG were used to identify outliers. A test of the homogeneity of intercepts across groups was performed to assure validity of the full bioassay model. When the slope-ratio assay showed a significant effect of drug use on the relationship between viral load and lnCD4+, a more comprehensive model using the actual drug use levels (days used or urinary cocaine concentration) was investigated by computing a multiple regression analysis using RNA viral load and interaction terms of RNA x DrugUse. This analysis avoids the arbitrary selection of stratification levels and produces a more general functional relationship. Finally, the interaction models were fit in multiple regression models where the variables of age, years since HIV diagnosis, and HIV treatment status were investigated as possible confounders.

4. RESULTS

A total of 122 subjects (80 HIV-1 positive, 42 HIV-) who had complete data on age, time since HIV infection, CD4+ counts, RNA, and reported crack use in the last 30 days were analyzed (see Table 1). The only statistically significant difference between HIV+ and HIV- groups is in the CD4+ counts (t=9.73, 120 df, p<0.0001). Similar data are shown for crack users and non-crack users within the HIV+ group in Table 2. The average urinary cocaine level among those reporting crack use is ten times that among those reporting no crack use (t=1.74, 78 df, p=0.043 one-tailed). Those reporting not smoking crack could have been either inhaling or injecting cocaine prior to the urine sample.

Within stratum linear regression analysis for the crack use group “None” (n=28) showed three observations judged to be outliers. The estimated slope changed moderately but the model fit went from R² = 0.15 to R² = 0.65 when these outliers were removed. The regression line within this stratum was lnCD4+ = 6.79 -5.03 x 10⁻⁶ RNA, R² = 0.65, p < 0.0001. Within stratum linear regression analysis for the “Less than daily” crack use group (n=54) showed one observation judged to be an outlier. The effect of this outlier was expressed as a strong influence on the estimated slope, which went from 7.3 x 10⁻⁶ to 11.5 x 10⁻⁶ when the observation was deleted. The regression line within this stratum was lnCD4+ = 6.79 -5.03 x 10⁻⁶ RNA, R² = 0.65, p < 0.0001. The within stratum linear regression analysis for the “Daily” crack use group (n=40) showed two observations judged to be outliers. The effect of these outliers was expressed as a strong influence on the estimated slope, which went from 0.8 x 10⁻⁶ to 32.4 x 10⁻⁶ when the observations were deleted, as well as a strong influence on the model fit, which went from R² = 0.05 to R² = 0.30. The regression line was lnCD4+ = 6.57 -32.37 x 10⁻⁶ RNA, R² = 0.65, p < 0.0001.

The independent within stratum analyses showed very different slopes and reasonably constant intercepts across strata. The full slope-ratio assay was then performed using 74 observations with complete and valid data from the HIV+ subjects and 42 values from the HIV- subjects. The assumption of common intercepts for the stratum-specific regression lines was assured by the non-significant F-test for intercepts in the Analysis of Variance for the slope-ratio assay (F₂,112 = 0.62, ns). The stratum slopes for crack use groups shown in Table 3 demonstrate that the jointly estimated parameters are very similar to those independently estimated within each stratum.

Although the distance between the crack use strata was approximately linear (None = 0, Less than daily = 15.1 ± 1.1, and Daily = 30 ± 0.09 days), the separation of the regression lines was not linear. This suggests the possibility that the joint surface describing lnCD4+ counts as a function of RNA and crack use in days is in fact non-linear, and to be described as a more complex interaction term that most likely involves RNA and cocaine use. To test this possibility a multiple regression using lnCD4+ as the dependent variable and RNA and the product RNA x (Days² Crack Use) as independent variables was fit. As shown in Table 4, both RNA and RNA x (Days² Crack Use) were statistically significant. The multiple regression parameters in Table 4 yield the equation lnCD4+ = 6.65 – (4.50x10⁻⁶ + 2.51x10⁻⁸ Days²) RNA. Thus the days of crack use per month potentiate the effect of RNA in the sense that a heavy crack user will have lower CD4+ counts for a given RNA viral load. These estimates yield almost exactly the slopes for constant crack use shown in Table 3, thus lending validity to the stratified analysis and the parallel lines assay. In order to assess the adequacy of this model, the so-called “population based” CD4+ values were calculated based on the average of 17.5 days crack use by HIV+ subjects (see Table 1). RNA values were aggregated into mean ± standard error by deciles of RNA and plotted against model estimates in Figure 1.

In order to assess whether the variables of age, time since diagnosis, and use of AZT are confounders, a multiple regression including these variables was run. The use of AZT was considered in by running analyses both including and excluding the HIV-control group. There was no effect on the models if AZT was included. The estimate in the complete model for RNA x (Days² Crack Use) was β₂ = -2.47x10⁻⁸, which confirms the relationship was not to be confounded by these variables.

The analysis of the potential effect modification based on the laboratory measured urinary cocaine levels is important since the crack use data were based on self reports. Using the same methods as those used to analyze the crack use strata, the strata defined by tertiles of urinary cocaine yielded similar results. The Analysis of Variance indicated a common intercept (F₂,59 = 2.81, p=0.0656), and the simultaneous slope estimates (n= 95) are shown in Table 3.

Similarly, a multiple regression using lnCD4+ as the dependent variable and RNA and RNA x (lnCocaine) as independent variables was fit. The linear interaction term was chosen because the stratum-specific slopes increased linearly with tertile of urinary cocaine. As shown in Table 4, RNA was statistically significant but RNA x (lnCocaine)
Table 1. Summary Statistics (Mean ± Std. Err.) by HIV Group

<table>
<thead>
<tr>
<th></th>
<th>HIV- (42)</th>
<th>HIV+ (80)</th>
<th>Total (122)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in Years</td>
<td>35.5 ± 1.2</td>
<td>37.1 ± 0.9</td>
<td>36.5 ± 0.7</td>
</tr>
<tr>
<td>Years Since Diagnosis</td>
<td></td>
<td>3.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>CD4+ (cells/µl)</td>
<td>1123 ± 46</td>
<td>507 ± 39</td>
<td>726 ± 40</td>
</tr>
<tr>
<td>HIV-1 RNA (10³ molecules/ml)</td>
<td></td>
<td>94.7 ± 28.2</td>
<td></td>
</tr>
<tr>
<td>Days Crack Use in Last Month</td>
<td>14.5 ± 2.1</td>
<td>17.5 ± 1.3</td>
<td>16.2 ± 1.1</td>
</tr>
<tr>
<td>Urinary Cocaine</td>
<td>76.5 ± 19.2</td>
<td>182.2 ± 48.0</td>
<td>150.2 ± 34.2</td>
</tr>
<tr>
<td>AZT Use</td>
<td></td>
<td>21.3% (n=17)</td>
<td></td>
</tr>
</tbody>
</table>

There were urinary cocaine values for 30 HIV- subjects and 69 HIV+ subjects.

Table 2. Summary Statistics (Mean ± Std. Err.) by Reported Crack Use Among HIV+ Subjects

<table>
<thead>
<tr>
<th></th>
<th>No Crack Use (16)</th>
<th>Crack Use (64)</th>
<th>Total (80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in Years</td>
<td>36.8 ± 1.8</td>
<td>37.2 ± 1.0</td>
<td>37.1 ± 0.9</td>
</tr>
<tr>
<td>Years Since Diagnosis</td>
<td>4.0 ± 0.5</td>
<td>3.6 ± 0.5</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>CD4+ (cells/µl)</td>
<td>476 ± 96</td>
<td>513 ± 42</td>
<td>507 ± 39</td>
</tr>
<tr>
<td>HIV-1 RNA (10³ molecules/ml)</td>
<td></td>
<td>73.4 ± 25.0</td>
<td>94.7 ± 28.2</td>
</tr>
<tr>
<td>Days Crack Use in Last Month</td>
<td></td>
<td>21.8 ± 1.1</td>
<td>17.5 ± 1.3</td>
</tr>
<tr>
<td>Urinary Cocaine</td>
<td>22.3 ± 14.6</td>
<td>215.7 ± 55.2</td>
<td>182.2 ± 48.0</td>
</tr>
<tr>
<td>AZT Use</td>
<td>31.3% (n=5)</td>
<td>18.8% (n=12)</td>
<td>21.3% (n=17)</td>
</tr>
</tbody>
</table>

Table 3. Simultaneous Slope Estimates by Crack Use and Urinary Cocaine Levels

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crack Use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>6.66</td>
<td>0.07</td>
<td>90.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>None</td>
<td>-4.65x10⁻⁶</td>
<td>1.03x10⁻⁶</td>
<td>-4.50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Less than Daily</td>
<td>-11.5x10⁻⁶</td>
<td>1.63x10⁻⁶</td>
<td>-7.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Daily</td>
<td>-34.20x10⁻⁶</td>
<td>6.97x10⁻⁶</td>
<td>-4.91</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ln(Cocaine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>6.58</td>
<td>0.08</td>
<td>79.37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1st Tertile</td>
<td>-5.69x10⁻⁶</td>
<td>1.25x10⁻⁶</td>
<td>-4.55</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2nd Tertile</td>
<td>-9.17x10⁻⁶</td>
<td>1.65x10⁻⁶</td>
<td>-5.57</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3rd Tertile</td>
<td>-13.4x10⁻⁶</td>
<td>4.55x10⁻⁶</td>
<td>-2.95</td>
<td>&lt;0.0041</td>
</tr>
</tbody>
</table>

Table 4. Multiple Regression Estimates by Crack Use and Urinary Cocaine Levels

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crack Use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept (β₀)</td>
<td>6.65</td>
<td>0.07</td>
<td>95.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RNA (β₁)</td>
<td>-4.50x10⁻⁶</td>
<td>9.30x10⁻⁷</td>
<td>-4.84</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RNA(Days² Crack Use) (β₂)</td>
<td>-2.51x10⁻⁸</td>
<td>3.99x10⁻⁹</td>
<td>-6.29</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Urinary Cocaine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept (β₀)</td>
<td>6.57</td>
<td>0.08</td>
<td>79.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RNA (β₁)</td>
<td>-5.44x10⁻⁶</td>
<td>1.35x10⁻⁶</td>
<td>-4.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RNA(ln(Cocaine)) (β₃)</td>
<td>-1.02x10⁻⁶</td>
<td>5.41x10⁻⁷</td>
<td>-1.88</td>
<td>&lt;0.0635</td>
</tr>
</tbody>
</table>

just failed to reach statistical significance (p=0.0635, two-tailed). A one-tailed p-value would of course be significant. The data in Table 4 yield the overall equation ln(CD4+) = 6.57 – (5.44x10⁻⁶ + 1.02x10⁻⁶ ln(Cocaine))RNA. As was shown for days of crack use, increased levels of urinary cocaine have a potentiating effect on RNA viral load in the sense that heavy cocaine users will have lower CD4+ counts for a given RNA viral load.

5. DISCUSSION

We demonstrated that CD4+ counts are lower for a given viral load as self-reported crack use or laboratory determined cocaine levels increase. We interpret these
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Table 5. Relative Potency of Crack Use to Reduce CD4+ Counts

<table>
<thead>
<tr>
<th>Crack Usage</th>
<th>Slope Ratio</th>
<th>95% CI</th>
<th>VL_{100}</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
<td></td>
<td>143,771</td>
</tr>
<tr>
<td>Less than Daily</td>
<td>2.47 ± 0.37</td>
<td>1.73-3.21</td>
<td>57,982</td>
</tr>
<tr>
<td>Daily</td>
<td>7.35 ± 1.50</td>
<td>4.35-10.35</td>
<td>19,536</td>
</tr>
</tbody>
</table>

observations to mean that an interaction between drug abuse and HIV infection exacerbates the effects of virus load on CD4+ cells, a finding confirmed by other studies. (15) Although one study of HIV-seropositive men and women did not find any differences in progression between the two genders, the study did show that crack-cocaine use and two HIV-related symptoms were related to progression to AIDS using both univariate and multivariate methods (51). Another multivariate analysis showed a short-term decline in CD4+ cells in HIV-1 seropositives among a sample of cocaine injecting African American (predominantly) males (15). The data established an inverse relationship between CD4+ cell counts and virus load.

However, prior studies have not found significant effects of drug abuse on HIV progression (25). There are several possible causes for the differences in our findings. Multivariate regression analysis incorporated within-individual correlation of the CD4+ counts. The cohort combines men and women injectors without any attempt at focusing on a specific ethnic group. In the same cohort another analysis showed that injection patterns whose frequency is correlated with drug ingestion was not similar importance for many populations in spite of several negative findings in other large-scale cohort studies.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

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