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Urinary 8-hydroxy-2′-deoxyguanosine, a Metabolite of Oxidized DNA, is not Elevated in HIV Patients on Combination Antiretroviral Therapy

SIMON PAUL[a,*], MIKHAIL B. BOGDANOV[b], WAYNE R. MATSON[c], LINDA METAKIS[b], JONATHAN JACOBS[a] and M. FLINT BEAL[b]

[a]*Department of Medicine, Division of Infectious Diseases, New York Presbyterian Hospital-Weill College of Medicine of Cornell University, New York, NY, USA;* [b]*Department of Neurology and Neuroscience, New York Presbyterian Hospital-Weill College of Medicine of Cornell University, New York, NY, USA;* [c]*ESA Inc., Chelmsford MA, USA*

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Mitochondrial toxicity of nucleoside analogues has been proposed to be the etiology of a range of side-effects from antiretroviral therapy of HIV infection. In this study, urinary 8-hydroxy-2′-deoxyguanosine (8OH2′dG), a metabolite of oxidized DNA, was measured to determine if antiretroviral therapy leads to oxidative damage to DNA. A cross-sectional study was carried out measuring urinary 8OH2′dG in three groups of HIV-infected patients: (1) antiretroviral medication naïve, (2) patients on antiretroviral medications without lipodystrophy and (3) patients on antiretroviral medications with lipodystrophy. Twenty-five patients were enrolled in each group. The mean spot urinary 8OH2′dG measurements per mg creatinine for the three groups were: antiretroviral naïve 4.27 ± 0.61 (ng 8OH2′dG/mg creatinine ± SEM), on antiretroviral medications without lipodystrophy 2.88 ± 0.26, and on antiretroviral medications with lipodystrophy 3.27 ± 0.30. The differences between the means of the three groups is not statistically significant ($p = 0.055$), and these results are not significantly different from reported values for healthy controls [A carbon column-based liquid chromatography electrochemical approach to routine 8-hydroxy-2′-deoxyguanosine measurements in urine and other biologic matrices: a one-year evaluation of methods. *Free Radical Biology and Medicine* **27** (1999) 647–666].

Keywords: Antiretroviral therapy; 8-hydroxy-2′-deoxyguanosine; Lipodystrophy; Mitochondria; HIV

INTRODUCTION

Nucleoside analogues used for the treatment of HIV infection and for viral hepatitis have led to a range of complications, including myopathy, cardiomyopathy, pancreatitis, peripheral neuropathy, and hepatic steatosis with lactic acidosis.[2] Recently, it has been proposed that lipodystrophy, a disfiguring complication of antiretroviral therapy and HIV infection, may also be a result of mitochondrial toxicity of nucleoside analogues.[3] These complications are believed to result from the inhibition of the mitochondrial DNA polymerase-γ by nucleoside analogues. This inhibition of mitochondrial DNA synthesis is hypothesized to lead to decreased number and function of mitochondria.[2] With nucleoside analogue treatment, elevated levels of peroxide production by mitochondria has been reported,[4] and abnormal beta-oxidation of fatty acids in nucleoside treated mice has been seen even when mtDNA levels were found to be normal.[5] In human and animal studies of antiretroviral therapy, biopsy studies have shown both decreased mitochondrial to nuclear DNA ratios,[6,7] and elevated levels of oxidized mitochondrial DNA.[4,8]

*Corresponding author. Address: Center for Special Studies, New York Presbyterian Hospital, 525 East 68th Street, Room F-24, New York, NY 10021, USA. Tel.: +1-212-746-4180. Fax: +1-212-746-8415. E-mail: sip2002@med.cornell.edu

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It is not known why some patients on nucleoside therapy develop complications due to mitochondrial toxicity, and others do not. In addition, prior to the development of complications, there is no marker available to identify patients that are developing toxicity. Previously it has been reported in both human and mouse studies that urinary excretion of the oxidized DNA metabolite 8OH2′dG is elevated by treatment with the single nucleoside AZT.[4] Urinary 8OH2′dG could provide an accessible marker for detecting the development of toxicity in patients on combination antiretroviral therapy.

In this study, we measured the level of 8OH2′dG in urine samples from HIV-infected patients. Antiretroviral naïve patients were compared with patients on combination antiretroviral therapy to determine if antiretroviral therapy leads to elevated production of this metabolite of oxidized DNA. In addition, to investigate the hypothesis that lipodystrophy is related to mitochondrial toxicity, we looked at patients both with and without lipodystrophy to determine if 8OH2′dG would be further elevated in patients with lipodystrophy.

METHODS

Patients

Three groups of patients were enrolled in this study: antiretroviral naïve HIV-infected patients, HIV-infected patients on antiretroviral therapy without lipodystrophy and HIV-infected patients on antiretroviral therapy with lipodystrophy. Lipodystrophy was assessed by both a patient and primary care provider survey, based on the published survey of Carr.[9] As patients with lipodystrophy were recruited, patients matched by age, sex and duration of antiretroviral therapy (if on therapy) were recruited for the other two arms of the study. The study enrolled 25 patients in each group, for a total of 75 patients. Demographic, laboratory and medication history for each patient was obtained from their medical record. Urine specimens were obtained from each patient. One aliquot of the urine sample was assayed for urine creatinine by the clinical biochemistry laboratory of New York Presbyterian Hospital. A second aliquot was diluted 1:2 with 0.2 M LiAcetate, 8% Methanol buffer pH 6.3 and stored at −70. All patients gave informed consent. The study was approved by the IRB of Weill Medical College of Cornell University, protocol #1000-460.

Measurement of Urinary 8-hydroxy-2′-deoxyguanosine

All samples were coded and analyzed in a blinded fashion. All samples were thawed together and run consecutively. Urinary 8-hydroxy-2′-deoxyguanosine (8OH2′dG) was measured by the previously described carbon column-based liquid chromatography method of Bogdanov.[1] Equipment and reagents used were those described in the published procedure.

Statistical analysis (ANOVA, ANCOVA and linear regression analysis) were performed using SPSS statistical software. Analysis of dependent variables was carried out for individual factors and multiple covariates as indicated in the results section.

RESULTS

Patient Characteristics

Patient demographics, clinical data and antiretroviral treatment history are summarized in Table I. The only statistically significant difference between the group's demographics was a slightly lower median age in the antiretroviral naïve group ($p = 0.045$). Current CD4 cell counts were not significantly different between the three groups, however, nadir CD4 counts were lower for the treatment groups compared with the antiretroviral naïve patients. Time on medication data includes time on mono- and dual-nucleoside regimens that some patients may have been on prior to beginning highly active antiretroviral therapy (HAART). Only one patient had been on HAART for less than one year (six months), a patient in the lipodystrophy group.

Body Habitus Changes

Both patients and physicians reported body shape changes of both atrophy and adiposity in the majority of cases in the lipodystrophy group. For lipodystrophy patients, 19 of 24 (data not available for one patient) reported the occurrence of both atrophy and adiposity, 4 only atrophy and 1 only adiposity. For physicians, for 13 of 24 of these same cases they reported both atrophy and adiposity, in 6 cases only atrophy and in 5 cases adiposity only. The mean score of both atrophy and adiposity is also presented in Table I.

Urinary 8OH2′DG

The urinary 8OH2′dG is presented as a ratio of urinary 8OH2′dG per mg creatinine in the urine, to correct for varying urine concentration. The results are presented in Fig. 1 for the three groups. The results for the three groups of HIV-infected patients were: antiretroviral naïve 4.27 ± 0.61 (ng 8OH2′dG/mg creatinine, ±SEM), on antiretroviral medication, no lipodystrophy 2.88 ± 0.26, on

TABLE I Demographic and clinical characteristics

	Antiretroviral naïve	On HAART no Lipodystrophy	On HAART with Lipodystrophy
Demographics:			
Age (mean, yrs)	38.4	43.5	43.2
Number of patients (total)	25	25	25
Male	15	18	14
African American	14	14	10
Hispanic	4	7	4
White	7	4	10
Other	0	0	1
Clincal Data:			
Nadir CD4 (median)	370	175	173
Current CD4(median)	370	440	385
HIV RNA Viral Load (median)	8,600	<400	<400
Lipoatrophy score (mean)*	0.12	0.12	5.4
Adiposity score (mean)*	0.02	0.02	2.4
Body Mass Index	26.6	24.8	25.8
Treatment History:			
Time since beginning aRV therapy (median, months)	0	49	42
Current Regimen:			
Protease inhibitor†	0	13	15
NNRTI†	0	15	11
Zidovudine	0	4	6
Stavudine	0	18	16
Lamivudine	0	14	18
Didanosine	0	6	5
Abacavir	0	7	1
Zalcitabine	0	0	0
Hydroxyurea	0	3	0

*Lipoatrophy is on a scale of 0–15; adiposity is on a scale of 0–12. †Four patients were on combined PI/NNRTI therapy.

antiretroviral medication with lipodystrophy 3.27 ± 0.30. The differences between the three groups was not statistically significant ($p = 0.055$, one way ANOVA). These results also did not differ significantly from the previously reported levels in adult male and female controls (male controls 3.68 ± 0.047, female controls 3.96 ± 0.038 reported in Ref. [1]). Patient body mass index (BMI), and plasma creatinine showed a statistically significant correlation with 8OH2′dG per mg creatinine values (Pearson correlation coefficients of -0.309, $p = 0.009$ for BMI and -0.301, $p = 0.009$ for plasma creatinine concentration). However, there was no significant difference in the mean BMI or plasma creatinine between the three patient groups. Including these factors in an analysis of covariance, did not demonstrate the patient treatment group to be significantly associated with the 8OH2′dG/mg creatinine values. The mean values of 8OH2′dG/mg dG/mg creatinine did not differ significantly by gender (males 3.4 ng/mg, females 3.6 ng/mg, $p = 0.8$) or show a significant correlation with age.

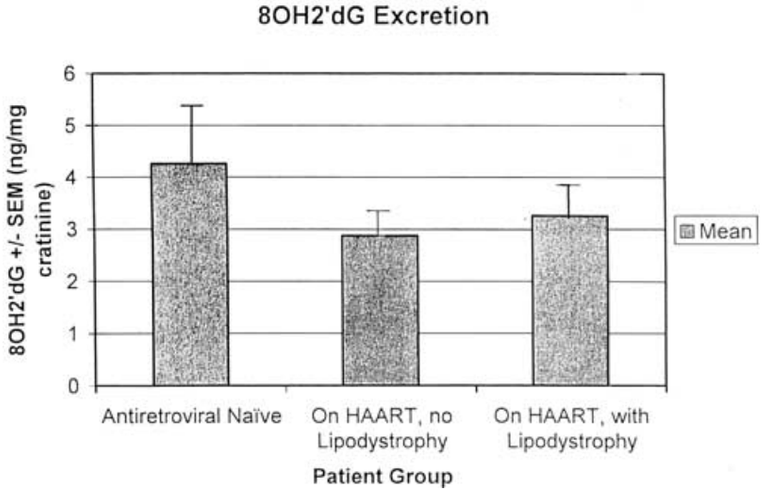

FIGURE 1 Urinary excretion of 8OH2′dG (ng per mg creatinine) ± SEM.

Other associations between 8OH2′dG/mg creatinine results in patient subgroups vs. antiretroviral naïve patients were also investigated. Subgroups compared with naïve patients included: patients on AZT based regimens (mean 3.20 ± 0.42 $n = 10$), D4T based regimens (3.04 ± 0.25 $n = 34$) PI based regimens (mean 3.36 ± 0.027 $n = 28$), Hepatitis C infected patients (mean 3.72 ± 0.57 $n = 29$). No statistically significant differences were found between these patient subgroups and the controls.

DISCUSSION

In this study, the relationship between combination antiretroviral therapy in HIV-infected patients and the excretion of 8OH2′dG, a metabolite of oxidized DNA, was investigated. No association was found between combination antiretroviral therapy and the level of excretion of 8OH2′dG in spot urine samples. Similarly, no association was seen between the presence of lipodystrophy, a complication occurring in patients on antiretroviral therapy that it has been hypothesized is due to mitochondrial toxicity, and excretion of 8OH2′dG.

The use of spot urine concentrations of 8OH2′dG and correcting for urinary concentration by expressing the results as a ratio of urine 8OH2′dG/mg creatinine, raises some potential problems. If creatinine excretion is elevated in patients with nucleoside analogue toxicity this would lead to underestimating the 8OH2′dG excretion by using ratios for comparisons (as opposed to 24 h collections). Similarly, creatinine excretion is affected by age, muscle mass, exercise and diet. However, there was no significant difference in the BMI, or plasma creatinine levels of the three treatment groups, and only a minimal difference in median age. Including these variables in an analysis of covariance did not demonstrate the three patient groups to have significantly different 8OH2′dG/mg creatinine results.

These results are surprising as it has been reported that AZT monotherapy leads to an increase in excretion of 8OH2′dG,[4] and elevated tissue levels of 8OH2′dG.[8] One might expect that patients on dual nucleosides would be at greater risk for mitochondrial toxicity than patients on monotherapy, and that they would have greater excretion of urinary 8OH2′dG. In fact, in the present study, levels of 8OH2′dG tended to be lower in patients on antiretroviral therapy, although the difference was not statistically significant ($p = 0.062$). These differing results, however, may not necessarily be in conflict. It is possible that with nucleoside monotherapy, mild mitochondrial toxicity leads to inefficient electron transport in mitochondria and elevated production of ROS. However, with combination nucleoside therapy a greater inhibition of mitochondrial function could potentially lead to decreased oxygen consumption and decreased production of ROS.

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