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Oxidative DNA Base Damage in Lymphocytes of HIV-infected Drug Users

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In the present study, we have studied the level of oxidative DNA base damage in lymphocytes of HIV-infected intravenous drug users (IDUs) and a seronegative control group. Chromatin was isolated from the lymphocytes and then analyzed by gas chromatography/isotope-dilution mass spectrometry with selected-ion monitoring (GC/IDMS-SIM). Significantly greater levels of four oxidatively modified DNA bases were observed in chromatin samples from the symptomatic HIV-infected patients than in those from the seronegative patients. These were 5-hydroxyuracil, 5-hydroxycytosine, 8-hydroxyadenine and 8-hydroxyguanine. In the case of 5-hydroxyuracil and 8-hydroxyguanine, a statistically significant difference was also found between the control group and the asymptomatic HIV-positive patients. These results suggest that oxidative stress may play an important role in the pathogenesis of acquired immune deficiency syndrome (AIDS), and that administration of antioxidant drugs to HIV-infected patients may offer protection against AIDS-related carcinogenesis.

Keywords: DNA base damage, gas chromatography/mass spectrometry, oxidative stress, reactive oxygen species, HIV/AIDS

INTRODUCTION

It is believed that patients infected with the human immunodeficiency virus (HIV) are under chronic oxidative stress.[1] Oxidative stress is defined as a condition characterized by an increased production of reactive oxygen species (ROS), which may include the highly reactive hydroxyl radical (•OH) and hydrogen peroxide.[2,3] ROS may be an important factor in mutagenesis and carcinogenesis.[2,4] Moreover, there is evidence that oxidative stress mediated by ROS is one of the causes of apoptosis.[5] ROS can cause DNA damage by modification of DNA bases.[6] Some of the modified DNA bases have been found to possess premutagenic properties.[7,8] Therefore, if they are not repaired within an appropriate time frame, they may contribute to carcinogenesis.[9] Alternatively these modified bases may trigger apoptosis of the cell. Recently, it was proposed that induction of apoptosis

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might play a central role in pathogenesis of acquired immune deficiency syndrome (AIDS).[10] It is well known that, when cellular DNA damage is not repaired, cells may be eliminated via apoptosis.[1,11,12]

The direct cause of apoptotic death of lymphocytes in HIV infected patients is not well known. Intravenous drug users (IDUs) are one of the groups most endangered by HIV-infection. In this study, we have studied the level of oxidative DNA base damage in lymphocytes of HIV-infected, symptomatic and asymptomatic IDUs with that of seronegative IDUs (control group). The aim was to see whether oxidative DNA base damage is increased in HIV-infected patients when compared with control HIV-free individuals.

MATERIALS AND METHODS

Blood samples were obtained from a control group of nine male HIV-seronegative IDUs (mean age 27 years, range 18–36 years) and from seventeen male HIV-infected IDUs (mean age 30 years, range 23–35 years). All of the patients were hospitalized in the Provincial Hospital of Infectious Diseases. They were on a stable diet for the weeks before and during this investigation. Both groups of the patients used heroin during approximately the same time period. HIV-infected patients (HIV+) were classified according to the Centers for Disease Control surveillance definition[13] [group IIa, (asymptomatic patients, $n = 9$), and group IIb (symptomatic patients $n = 8$)]. The mean CD4+ cell counts in asymptomatic patients and in symptomatic patients were 527/mm^3 (range 364–861/mm^3) and 146/mm^3 (range 91–247/mm^3), respectively. None of the patients had malignancies or signs of any infection. Chromatin was isolated from the lymphocytes and then analyzed by gas chromatography/isotope-dilution mass spectroscopy with selected-ion monitoring (GC/IDMS-SIM) as described.[14,15]

RESULTS AND DISCUSSION

Using GC/IDMS-SIM technique, the following modified DNA bases were identified and quantified in chromatin samples isolated from lymphocytes: 5-hydroxyuracil (5-OH-Ura), 5-hydroxycytosine (5-OH-Cyt), 8-hydroxyadenine (8-OH-Ade) and 8-hydroxyguanine (8-OH-Gua). Considerable individual differences were observed in the levels of modified bases found in chromatin isolated from lymphocytes of the control group as well as the HIV-infected patients (Figure 1). Similar variations in the modified base levels were observed by us[16–18] and others[19] in DNA isolated from lymphocytes of cancerous and non-cancerous tissues of patients with different types of cancer. The levels of four modified DNA bases in the chromatin samples isolated from lymphocytes of the symptomatic patients were found to be significantly greater than those in chromatin samples from control patients (Figure 1). Table I shows the mean values and standard deviations calculated from the values in Figure 1. The levels of 5-OH-Ura and 8-OH-Gua were also significantly greater in asymptomatic HIV-infected patients than those in control patients (Figure 1, Table I). The greater levels of modified DNA bases may be a result of the reaction of endogenously produced ROS with cellular DNA.[2] Since there is no evidence that AIDS is associated with a reduced ability to repair DNA damage, the observed increase could be a result of higher production of ROS in lymphocytes of HIV-infected patients. A wide variety of evidence supports the notion that oxidative stress is involved in the progression of AIDS. An excess of hydrogen peroxide combined with a deficiency in antioxidant enzymes, catalase and glutathione peroxidase, may lead to the overproduction of $^{\bullet}$OH,[1] which in turn may be responsible for oxidative DNA damage.[20] Typical products of reactions of $^{\bullet}$OH with DNA bases were detected in this work. These modified bases may possess premutagenic properties.[7,8] Their increased production in lymphocytes of HIV-infected patients

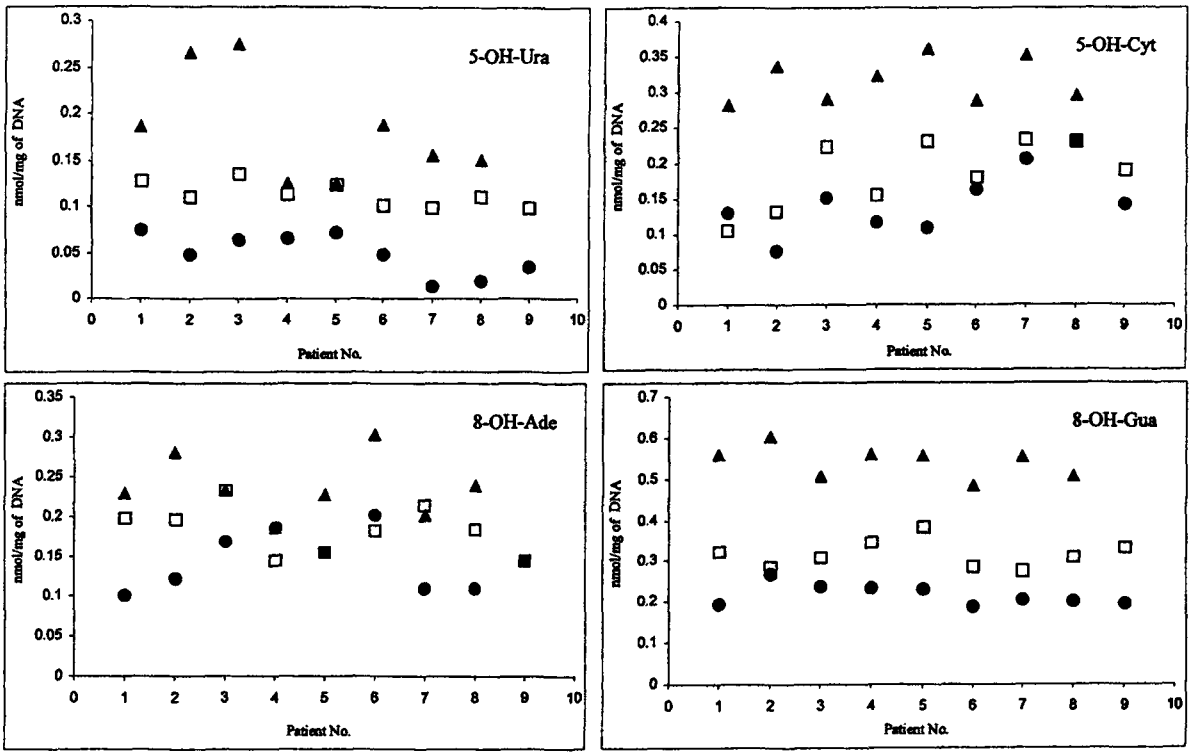

FIGURE 1 Levels of four modified DNA bases in lymphocytes of individual HIV negative (HIV−) (●), positive IDUs: HIV+ (IIa) (asymptomatic patients) (□), and HIV+ (IIb) (symptomatic patients) (▲). The same individual has the same number in each plot and the numbers were given arbitrarily. One nmol of a lesion/mg of DNA corresponds to approximately 32 lesions/10^5 DNA bases.

TABLE I Levels of modified DNA bases (nmol/mg of DNA) in lymphocytes of HIV negative (HIV−) and positive IDUs [HIV+ (IIa) (asymptomatic patients) and HIV+1 (IIb) (symptomatic patients)]

Modified base	HIV−	HIV+ (IIa)	HIV+ (IIb)
5-OH-Ura[a,b,c]	0.048 ± 0.022	0.113 ± 0.013	0.184 ± 0.040
5-OH-Cyt[b,c]	0.147 ± 0.048	0.187 ± 0.048	0.316 ± 0.031
8-OH-Ade[b,c]	0.144 ± 0.036	0.184 ± 0.031	0.238 ± 0.038
8-OH-Gua[a,b,c]	0.217 ± 0.028	0.316 ± 0.034	0.541 ± 0.040

The values represent the mean ± standard deviation and were calculated from the values in Figure 1. One nmol/mg of DNA corresponds to 32 lesions/10^5 DNA bases. The data were analyzed by the Student t-test ($p < 0.05$)
[a]significant difference between HIV− and HIV+ (IIa);
[b]significant difference between HIV+ (IIa) and HIV+ (IIb);
[c]significant difference between HIV− and HIV+ (IIb).

may be related to malignancies associated with AIDS, as HIV-infection predisposes an individual to several neoplasias, especially non-Hodgkin's lymphoma of B-cell origin.[21–23] Recently, increased levels of oxidatively modified DNA bases were found in cancerous and precancerous tissues, suggesting the involvement of oxidative DNA damage in cancer development.[16,17]

It has been proposed that apoptosis initiated by oxidative stress is the direct cause of lymphocyte loss in patients infected with HIV.[10,24] However, the mechanisms, which trigger apoptosis, have not been elucidated. One of the mechanisms for apoptotic death of cells may be unrepaired DNA damage. There is evidence that oxidative DNA damage induces apoptosis in murine T-cell hybridoma and in lymphocytes.[25,26] Oxidatively modified DNA bases, which were found to have greater levels in HIV-infected patients than in control patients, may contribute to apoptosis. In this context, a recent study demonstrated that lymphocytes of HIV-infected individuals even at very early stages of infection were more

susceptible to oxidative stress-mediated apoptosis than lymphocytes isolated from a control group.[1]

In conclusion, significantly greater levels of oxidatively modified DNA bases were found in symptomatic HIV-infected IDUs than in seronegative IDUs. We postulate that the observed greater levels of oxidative DNA base damage may be one of the variables responsible for the apoptotic death of lymphocytes and carcinogenesis related to HIV-infection. This is the first study of oxidative DNA base damage in lymphocytes isolated from HIV-infected patients. The results support the notion that oxidative stress may play an important role in AIDS pathogenesis and the use of antioxidant drugs in the therapy of HIV-infected patients may offer protection against AIDS-related carcinogenesis and apoptosis.

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