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Serum Malondialdehyde Correlates with Therapeutic Efficiency of High Activity Antiretroviral Therapies (HAART) in HIV-1 Infected Children

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Serum malondialdehyde (MDA) levels are increased in human immunodeficiency virus (HIV)-infected children, as it happens also in infected adult individuals. Introduction of high activity antiretroviral therapy (HAART) has promoted an intense decline in morbidity and mortality of these patients. Here we present data on the effect of HAART on serum MDA of HIV+ children and compare them with levels prior to HAART. MDA levels reflect, as other markers do, the HAART-induced clinical improvement and probably also the pro-oxidant/antioxidant side effects of the different drugs used. The results herein allow the proposal of including serum MDA levels as an additional parameter for the clinical management of HIV+ children.

Keywords: Malondialdehyde; Oxidative stress; HIV; Children; HAART

INTRODUCTION

It has been repeatedly reported that HIV infection is associated with oxidative stress, in view of the increased serum concentrations of the lipid peroxidation product malondialdehyde (MDA), and the decrease of antioxidant defences such as glutathione in HIV infected patients.[1–5] This oxidative burden can potentiate disease progression through activation of NF-κB, a cytosolic protein that, when activated, stimulates viral replication.[6,7] In this sense, we have previously reported that serum MDA levels are increased in HIV infected children, when compared with controls,[8] and proposed the use of these levels as additional criteria for the clinical follow up of paediatric patients.[9,10] Other markers more frequently used in clinical practice: T-CD4+ lymphocyte count (immunological status), plasma viral load (virological status) and the classification proposed by the CDC of 1994 (clinical status),[11–14] show different shortcomings when applied to paediatric populations, in view of the immaturity of the immune system in children, that leads to a high viral load in children under 5 years of age.[15] These facts make it necessary to seek for additional analytical markers for the clinical management of HIV-infected children.

In the latest years, a relevant decline of the morbidity and mortality of this disease has been observed due to the use of potent combined therapies named high activity antiretroviral therapies (HAARTs). These therapies have led to a decrease of viral load, and a quantitative and qualitative improvement of the immune function in these patients, specially T-CD4+ lymphocyte count, having as a consequence a decrease of infectious complications and a global clinical improvement.[16–19] Some of these antiretroviral drugs may

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TABLE I Serum MDA in control and HIV-infected children, before and after the use of HAART

Group	*n*	MDA μ(μM)	*p* vs control
Control	38	0.349 ± 0.133	–
HIV+Pre-HAART	22	0.558 ± 0.223	0.0003
HIV+Post-HAART	18	0.432 ± 0.207*	0.039

*$p = 0.037$ vs pre-HAART.

have specific side effects related to oxidative damage, as it has been suggested in animal experiments.[20] Here we show the apparent effects of the different drug combinations of HAART on MDA levels in children, to test this latter possibility, and propose that this analytical parameter may also be used to assess therapeutic efficiency in these patients.

PATIENTS AND METHODS

Forty serum samples from 17 HIV-infected children were used throughout the study (from February 1995 to January 2000). Twenty-two of them before the use of HAART (non-treated or treated only with one or two nucleoside reverse transcriptase inhibitors (NRTI)) and 18 after HAART (two NRTIs together with one or two protease inhibitors (PI), or two NRTIs together with one non-NRTI, or two NRTIs together with one PI and one non-NRTI) as recommended by CDC.[21] They were grouped according to the clinical status of the children[12] at the moment when the blood sample was obtained, as follows: 11 asymptomatic (stage N), 11 with mild (stage A), 11 with moderate (stage B), and seven with severe symptoms (stage C). Thirty-eight serum samples were obtained from healthy children attending the emergency room and requiring blood sample withdrawal for control tests. Informed consent was obtained from parents or legal tutors of each child. This study was approved by the Ethics Committee of our institution.

Serum MDA was assayed basically as previously described.[8,22] Data are expressed as means ± standard deviations. Statistical significance (p) between the different groups was established with the Student's t-test, and linear correlation with the Pearson's coefficient (r) and its significance (P).

RESULTS

MDA levels were significantly lower in HAART-treated children than in the non-HAART group (0.432 ± 0.207 vs 0.558 ± 0.223 μM, respectively, $p = 0.037$). Both groups presented MDA values significantly higher than the control group (0.349 ± 0.133 μM) (*cf.* Table I for statistical significance).

In order to be able to correlate MDA levels with the clinical stage, we assigned each of the CDC classification possibilities: N, A, B and C,[12] values 1–4, assuming the same clinical "distance" between each other, as we had previously described.[10,23] Mean values of MDA levels for each clinical group showed a very good positive correlation with the clinical status, when only pre-HAART samples were considered (Fig. 1, open symbols); however, the correlation coefficient using only the post-HAART samples looses significance (Fig. 1, closed symbols) when compared with the correlation of the pre-HAART samples ($r = 0.759$, $P = 0.241$ vs $r = 0.978$, $P = 0.022$, respectively).

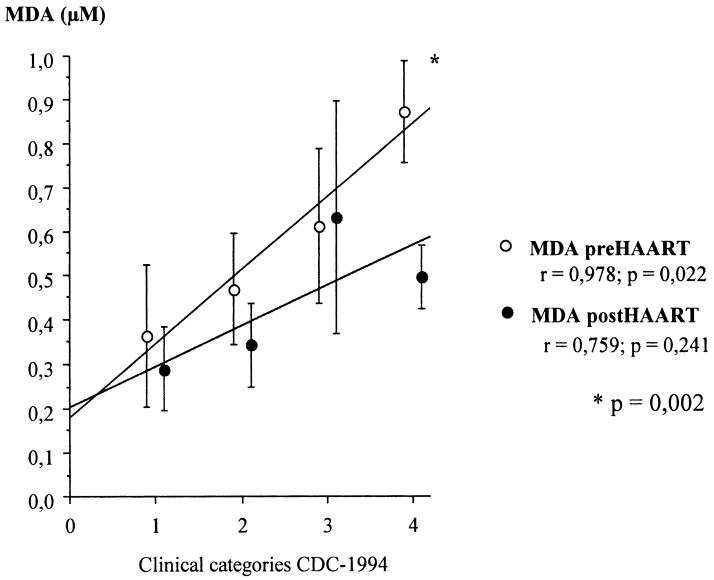

FIGURE 1 Linear correlation between MDA levels and stages of the clinical classification from CDC (1:N, 2:A, 3:B, 4:C) in samples of HIV-infected children before and after HAART. *p shows the significance of the difference between MDA levels in stage C before and after HAART.

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TABLE II Serum MDA concentration in samples of untreated HIV-infected children and HIV+ children treated with different NRTIs (alone or in combination with other drugs)

Drug used	*n*	MDA (μM)	*p* vs untreated	Significance
None (Untreated)	8	0.566 ± 0.255	–	–
AZT	17	0.504 ± 0.208	0.244	N.S.
ddI	12	0.477 ± 0.227	0.188	N.S.
D4T	13	0.453 ± 0.232	0.131	N.S.
3TC	12	0.411 ± 0.174	0.044	$p < 0.05$

The possible influence on MDA levels of single drugs, specially NRTIs, or the different therapeutic combinations where they are used, was also tested. Serum samples of children treated with NRTIs, i.e. zidovudine (AZT), didanosine (ddI), stavudine (d4T) and lamivudine (3TC) (alone or in combination with other drugs), show MDA levels somewhat lower than the samples of untreated children, whereas the only significant difference ($p = 0.044$) was observed with 3TC (Table II).

DISCUSSION

The presented results confirm the previous finding that serum malondialdehyde can be used as a parameter for the clinical management of HIV-infected children also after the HAART era. The correlation between serum MDA levels and the clinical CDC staging of the children shown in Fig. 1, also confirms our previous reports.[10,23] We propose that the loss of significance of this correlation that occurs when post-HAART samples are used, is due to the imposed immobility criteria of the CDC classification that does not allow turning back after a clinical improvement.[12] However, MDA levels reflect the clinical improvements due to therapy; as an example, children on stage C prior to HAART presented severe clinical manifestations and higher MDA values than after HAART, within a long asymptomatic period of time (0.872 ± 0.115 vs $0.498 \pm 0.072\,\mu M$, respectively; $p = 0.002$). In this sense, it has been recently established that MDA (and possibly other lipid peroxidation products such as 4-hydroxy-nonenal) exert a direct effect on the expression of aldehyde reductase and some other detoxification enzymes via an increase in peroxide levels.[24] Whether the decrease in MDA is the consequence of a decreased oxidative burden, or the effect of a higher rate of MDA catabolism by aldehyde reductase, is an open question an deserves further investigation.

The nucleoside analogues (NRTIs) have been associated with mitochondrial toxicity via their effect on mitochondrial polymerase gamma, that causes cellular respiratory dysfunction and clinical symptoms such as miopathy, neuropathy, lactic acidosis and even lipodistrophy.[25] Experimentally, it has been recently proposed that AZT could act as a pro-oxidant in view of the observed damage in mitochondrial DNA of acute treated animals.[20] Our data do not directly support these findings since no increase in MDA levels is observed in the samples of children treated with AZT alone or in combination, when compared with the rest of the samples (data not shown), or with samples of untreated children (Table II). However, the fact that the observed therapy-induced clinical amelioration, accompanied by a decrease in MDA, could be responsible for the masking of this toxic effect of AZT, cannot be excluded, and would fit with the hypothesis.[20] Moreover, 3TC, the only sulphur-containing drug of the ones used in the present study, is the only one showing statistically significant lower MDA levels in the samples treated with this drug alone or in combination, when compared with samples of untreated children (*cf*. Table II). Since no grading of clinical efficacy has been established for the different NRTIs, it might be possible that these differences in MDA levels could be accounted for by the different pro- or antioxidant features of the NRTIs' molecules. Preliminary data confirm that this proposed beneficial effect of 3TC, can also be observed in viral load when compared with AZT (grouped as in Table II) (3.37 ± 1.27 vs 4.31 ± 1.15 log viral load, respectively, $p < 0.013$). *In vivo* and *in vitro* experiments are in progress to proof this hypothesis.

The results herein allow the conclusion that assays of MDA levels could be used in the follow up of HIV-infected children as a marker of therapeutic response, because of their significant decrease with HAART, and because of their capability to reflect clinical improvements with HAART (which does not happen with the clinical classification from CDC).

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