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A Color Doppler Ultrasound-based Comparative Study between Stavudine and Non-stavudine Regimens in the Onset of Vascular Lesions in HIV-1-positive Patients

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Abstract. *Background: The present study aimed to investigate the role of stavudine in the onset of premature vascular lesions using an ultrasound color Doppler evaluation of the carotid vessels. Patients and Methods: A total of 266 patients were evaluated: 149 were treated with stavudine (group I) and 117 without stavudine (group II). Results: Of the patients in group I, 41% exhibited vascular lesions vs. 26% in group II (p=0.0103). The two groups were further divided into subgroups Ia (stavudine and proteinase inhibitor, PI), Ib (stavudine and non-nucleotidic reverse transcriptase inhibitor, NNRTI), IIa (PI, without stavudine) and IIb (NNRTI without stavudine). A higher prevalence of lesions emerged in group Ia, while group IIa were at higher risk of developing vascular lesions than groups Ib and IIb. Conclusion: Although stavudine per se does not seem to determine damage of the epiaortic vessels, the association of a PI with stavudine is related to a significantly higher rate of lesions.*

Various studies have found stavudine to contribute to the development of lipoatrophy (1), hyperlactatemia (2) and dyslipidemia (3, 4). This evidence determined a progressive abandonment of this molecule as a part of antiretroviral combination therapies. Nevertheless, to date, there is no evidence of a direct involvement of the drug in the onset of vascular lesions, an emerging issue among HIV-infected patients.

Color Doppler ultrasound is a well-established method for measuring the degree of atherosclerosis. Furthermore, the examination can be conducted early in the development of atherosclerosis and can be used to detect the progression of the lesions. In previous reports within the PREVALEAT study (premature vascular lesions and antiretroviral therapy), a higher than expected prevalence of premature carotid lesions on color Doppler ultrasonograpy of the epiaortic vessels was observed in HIV-1-infected patients, especially when treated with protease inhibitor (PI)-including regimens (5). The aim of the present study was the evaluation of the role of stavudine in the onset of these lesions.

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Patients and Methods

A total of 266 HIV-1 patients were evaluated. These comprised 149 in treatment with antiretroviral regimens including stavudine (group I) and 117 in treatment with regimens not including stavudine. Patients affected with hypertension were excluded from the study. The main patient characteristics are reported in Table I. HIV-1 seropositivity had been documented for a median period of 8 years (range 1-19 years). All patients were in therapy for a median period of 25 months (range 10-84 months). In group I, patients were treated for a median period of 27 months (range 12-84 months). In group II, the median treatment period was 22 months (range 10-84 months). In group I, 83 patients were treated with PI-based regimens; the remaining 66 were treated with non-nucleosidic reverse transcriptase inhibitors (NNRTI)-based regimens. In group II, 53 patients were treated with PI-based regimens, 64 with NNRTI-based regimens. All the treated patients had been in stable therapy for at least 12 months.

All patients were subjected to ultrasonography of the epiaortic vessels using a power color Doppler instrument with 7.5 MHz probes (ACUSON sequoia 512). Characteristics of the intima, pulsation index, resistance index, minimal speed, peak speed and mean speed were evaluated. An intima media thickness (IMT) of >1 mm was considered to be pathological. Atherosclerotic plaques, if present, were described. Ultrasonography was performed by physicians specifically trained in the examination of carotid vessels with a 10-year experience in the color Doppler ultrasound technique and at least 1,000 documented epiaortic examinations. They were blinded to the patient's antiretroviral therapy. Moreover, during the study, periodic meetings were held using filmed reports aimed at the comparison and standardization of the technique.

Patients were submitted to the investigation in a supine position after at least 10 minutes of acclimatization in a comfortable room. Patients were informed that the investigation was non-invasive. The

Table I. *Epidemiological characteristics and risk factors of HIV+ patients subjected to ultrasonographic investigation of the epiaortic vessels in two therapeutic regimens.*

	Group I Pts treated with stavudine		Group II Pts not treated with stavudine	
Characteristic	No.	%	No.	%
	149		117	
Gender				
Male	111	74.0	85	73.0
Female	38	26.0	32	27.0
Risk factor				
Intravenous drug addiction	62	42.1	47	43.0
Homosexual	44	29.3	35	32.0
Heterosexual	29	19.3	15	15.0
Unknown	14	9.3	11	7.0
Age (years; median, range)	38	11-67	37	23-64
Time from diagnosis of HIV infection (years)	8	1-19	6	1-19
Period of therapy (months)	27	11-84	22	10-84
CD4+ count (cells/mm^3)				
<200	85	57.0	12	10.3
200-500	16	10.8	61	52.7
>500	48	32.2	44	37.0
Viral load (copies/ml)*				
<80	69	46.7	70	60.0
80-30,000	60	40.0	41	35.0
30,000-100,000	12	8.0	2	1.8
>100,000	8	5.3	4	3.2
Stage of disease**				
A	84	56.8	77	66.6
B	16	10.6	9	7.4
C	49	32.6	31	26.0
Familial history of CVD	70	47.3	58	50.0
Cigarette smoking	104	69.3	81	70.0
Alcohol abuse	15	10.0	8	7.0
Sedentary life	118	78.6	81	70.0
Active drug addiction	3	2.0	0	0.0
Hypertriglyceridemia (>200 mg/dl)	59	39.3	30	26.0
Hypercholesterolemia (>200 mg/dl)	61	40.6	40	35.0
Hyperglycemia (>110 mg/dl)	17	11.3	8	7.5

*Nucleic acid sequence-based amplification (NASBA) test (Organon Teknika, Toronto, Canada); **revised CDC classification (Atlanta 1993).

common, internal and external carotid vessels were examined in the short and long axis. The percentage of stenosis was always quantified by calculating the stenosis areas in the short axis using a strong magnification. This was intended to correctly distinguish the real lumen from plaques markedly hypoechoic with the color or the power Doppler. The speed measurements were performed at an 45˚-

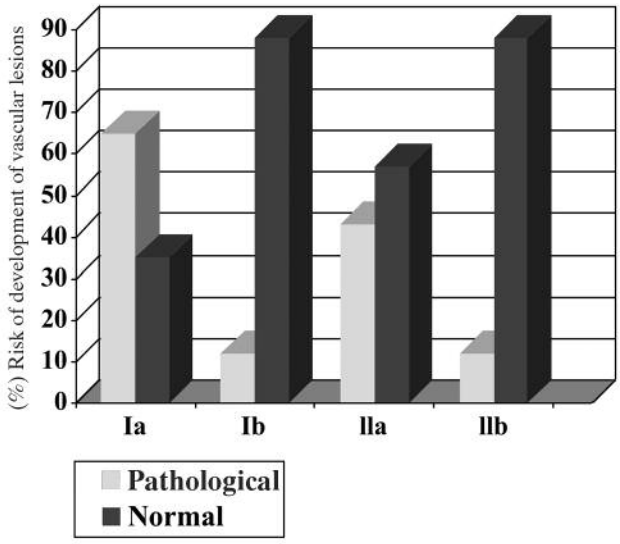

Figure 1. *Role of stavudine in protease inhibitor and nonprotease inhibitor regimens. Ia, stavudine in protease inhibitor regimen; Ib, stavudine in non-nucleosidic reverse transcriptase regimen; IIa protease inhibitor regimen without stavudine; IIb, non-nucleosidic reverse transcriptase regimens without stavudine.*

60˚ inclination with respect to the lumen. The morphological investigation of the plaque was performed using both ultrasonography and the ultrasound power color Doppler in order to better characterize the profile of the plaque and the IMT (6-9). None of the patients was subjected to angiography because of poor patient compliance with the invasive procedure. Moreover, previously published literature confirms that the technique used here is the gold standard for investigation of carotid plaques (10).

Risk factors for cardiovascular diseases were evaluated, such as familial history (angina, myocardial infarction, cerebral stroke, transitory ischemic attack), sedentary life (<1 hour/week of sport activity), cigarette smoking, alcohol consumption (>80 g/day), active drug addiction, hyperglycemia (>110 mg/dl), hypercholesterolemia (>200 mg/dl), hypertriglyceridemia (>200 mg/dl); in addition, risk factors for HIV-1 infection including stage of the disease (Center for Disease Control of Atlanta classification 1993), CD4 cell count, viral load and antiretroviral therapy were assessed.

Statistical analysis was performed using the chi-square test (χ^2) and Fisher's exact test to evaluate the association between the vascular lesions and the antiretroviral regimens. Moreover, to simultaneously estimate the influence of all variables analysed for the presence/absence of vascular lesions, logistic regression analysis was performed. M-L chi-square test and Hotelling test were used when necessary.

The Ethics Committee of the hospital approved the study and the patients provided informed consent. No conflict of interest was reported for any author.

Results

Of the patients included in group I, 41% exhibited lesions of the epiaortic vessels. In detail, 30 (49%) of them had a pathological IMT, 20 (31%) a pathological IMT and plaque, and 12 (20%) had only plaques. The median pathological IMT on the right side was 1.12 mm/mm (range 1.01-4.7 mm/mm). On the left side, the median pathological IMT was 1.21 mm/mm (range 1.01-3 mm/mm) In group II, 26% of the patients developed lesions. Of these, 11 (33%) had a pathological IMT, 13 (42.5%) had pathological IMT and plaques, and 7 in this group (24.5%) had only plaques. The median pathological IMT on the right side was 1.2 mm/mm (range 0.70-2.33 mm/mm). On the left side the median pathological IMT was 1.1 mm/mm (range 1.10-2.6 mm/mm). On statistical analysis, the differences between the two groups were significant (χ^2 6.585 p=0.0103).

To better evaluate the simultaneous role of the therapy with stavudine and PI in developing vascular lesions, we further divided the two groups of patients into two other subgroups each: Ia (therapy including stavudine and PI), Ib (therapy including stavudine and NNRTI), IIa (therapy including PI, but not stavudine) and IIb (therapy including NNRTI without stavudine). The comparison among these subgroups revealed a significantly higher risk for the development of vascular lesions in group Ia with respect to Ib (65% *vs.* 12%; χ^2: 42.408, p<0.0001), IIa (65% *vs.* 43%, χ^2: 6.181, p<0.0129) and IIb (65% *vs.* 12.5%; χ^2: 40.934, p<0.0001). No statistically significant differences emerged among groups Ib and IIb (χ^2: 0.004, p=0.9476), while group IIa exhibited a higher risk with respect to groups Ib (χ^2=14.925, p<0.0001) and IIb (χ^2=14.210, p<0.0002). All results were confirmed with Fisher's exact statistical analysis (Figure 1). Group Ib seems to have the lowest rate of lesions (12% *vs.* 12.5% of IIb group), but this paradoxical protective effect of the use of stavudine in regimens including NNRTI or 3 NRTI was no longer apparent when the logistic regression was applied. In fact, using the Wald test, the only significant effects were linked to the use of PI, wich confers a four-fold risk of vessel lesions in the absence of stavudine, and a five-fold risk when administered together with stavudine. Among the independent risk factors, the age of the patients represents a major determinant, conferring an adjunctive 10% risk per year of age. Regarding the other independent risk factors, male sex and hypercholesterolemia represent a significant risk factor with a five-fold increase in probability of lesions, but only in association with each other. Regarding the stage of the disease, CDC stage B and C were associated with an increasing risk of lesions. These statistical data confirm our observations in previous studies (5).

Discussion

HIV-1-positive patients are, nowadays, considered at increased risk for cardiovascular diseases, especially those submitted to highly active antiretroviral therapy (HAART) (11). Various studies have hypothesised a direct involvement of PIs, while no data are available on the role of stavudine in the onset of cerebro- and cardiovascular diseases. Actually, since stavudine

is capable of causing a metabolic disturbance (3, 4), it could theoretically predispose patients to atheromatous lesions of the vascular walls. Moreover, the same lipodystrophy syndrome associated with the use of stavudine could determine altered circulating levels and adipose tissue mRNA expression of proinflammatory cytokines, interleukin-6, tumour necrosis factor alpha and adiponectin (12). Proinflammatory cytokines and adiponectin, which are secreted by adipose tissue, regulate fat metabolism, insulin sensitivity and adipose cell apoptosis. Increased cytokine and reduced adiponectin secretion and expression may contribute not only to adipose tissue loss, but also to insulin resistance, a well-known condition associated with endothelial inflammation and atherothrombosis (13).

Color Doppler ultrasound, which incorporates the ultrasonographic and Doppler methods, is a safe, inexpensive and fast technique which allows an accurate evaluation of the vascular vessel. Moreover, the images obtained can be highly magnified. Using this procedure, we observed in the present investigation that the use of a regimen including stavudine seems to be associated with the presence of epiaortic lesions. But if we further divide the patients into four groups (treated with PI and stavudine, PI but not stavudine, NNRTI with stavudine and NNRTI without stavudine), we can observe a statistically significant presence of lesions at ultrasonographic investigation in the group of patients treated with PI and stavudine.

Conclusion

Although stavudine including regimens *per se* do not seem to determine damage of the epiaortic vessels, the association of a PI-including regimen with stavudine is related to a statistically significant higher number of these lesions.

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