

Study of Vascular Reactivity in HIV Patients whether or not Receiving Protease Inhibitor

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Summary

Background: A great number of HIV-infected patients using antiretroviral drugs develop endothelial dysfunction and atherothrombosis, which lead to a high medical and social burden. Thus, it is important to identify pathophysiological mechanisms involved with the endothelial function in these patients, so that early intervention can be made to avoid disease progression.

Objective: To evaluate endothelial function using endothelium-dependent and independent vasodilation in HIV-positive patients and in a control group.

Methods: A total of 27 HIV-positive patients and 16 controls were evaluated. Endothelium-dependent (reactive hyperemia) and independent (SL nitroglycerine) vasodilation of the brachial artery was used to evaluate the endothelial function.

Results: HIV-positive patients receiving protease inhibitors (PI) showed significantly lower endothelium-independent vasodilation than the HIV-negative ($p=0.020$) and HIV-positive without PI ($p=0.034$) subgroups.

The change in brachial artery diameter during active hyperemia was not statistically significant in any subgroup. Multiple linear regression analysis showed that only PI was associated with the relative delta of brachial reactivity to vasodilator in HIV-positive patients at 60s and 90s.

Conclusion: HIV-positive patients receiving PI presented endothelium-independent dysfunction when compared to HIV-positive patients not receiving PI and to the control group. (Arq Bras Cardiol 2009; 93(3) : 340-346)

Key Words: Endothelial dysfunction; vascular Doppler; HIV.

Introduction

By the end of the 1980's, Human Immunodeficiency Virus (HIV)-infected patients had short survival¹. In contrast, in the early 21st century, there was an increase in the life expectancy of these patients thanks to the impact of the treatment with new antiretroviral drugs - the Highly Active Antiretroviral Therapy (HAART)^{2,3}. HAART improves the prognosis of HIV-infected patients by reducing the incidence of opportunistic infections, hospitalizations and mortality⁴.

Several factors contribute to the increase in cardiovascular disease risk in HIV patients, such as chronic inflammation due to the viral infection, PI action, and interaction with traditional risk factors⁵. Therefore, the predisposition to atherosclerosis results from cumulative exposure to the virus and from metabolic changes secondary to the use of PI, thus

determining an unfavorable metabolic profile characterized by hypertriglyceridemia, low HDL-c and insulin resistance observed in 25 to 60% of the patients⁶.

PIs are currently considered independent cardiovascular risk factors and are associated with an increase by 26% in the rate of acute myocardial infarction (AMI) per year of exposure in the patients who had started treatment for at least 4 to 6 years⁷. In 1998, the first two cases of premature coronary artery disease were demonstrated in two young HIV-positive patients receiving PI⁸.

Several studies have demonstrated that endothelial dysfunction occurs in the early stages of insulin resistance found in patients receiving HAART. Hypoadiponectinemia is one of the factors responsible for lipodystrophy and metabolic disorder in HIV patients receiving PI^{9,10}. Endothelial dysfunction is a premature event in the development of atherosclerotic lesions and may be easily measured using non-invasive ultrasound study of the brachial artery flow¹¹. The mechanisms that lead to endothelial dysfunction in HIV-positive patients receiving PI remain not fully understood.

The objective of this study was to evaluate the endothelium-dependent and independent function by means of the non-

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invasive method of flow-mediated vasodilation of the brachial artery (DILA), using ultrasound study in HIV-positive patients with and without HAART. DILA was compared in three groups: HIV-infected patients not receiving PI, HIV-infected patients receiving PI, and HIV-negative controls.

Methodology

A total of 43 patients of both genders aged between 20 and 55 years who had undergone clinical and laboratory assessment and the 1-day protocol for non-invasive ultrasound study of the endothelial function were included in the study.

Group I included 15 HIV-positive patients in chronic use (> 2 years) of PI, and group II included 12 HIV-positive PI-naïve patients. A control group of 16 HIV-negative volunteers of both genders was also evaluated.

Exclusion criteria were the presence of coronary artery disease; cerebrovascular disease; peripheral vascular disease; use of contraceptives; ventricular dysfunction (left ventricular ejection fraction < 45% using Simpson's rule); plasma creatinine > 1.5 mg%; neoplastic diseases; and thyroid diseases.

Data from the patient's clinical history included age, gender, weight (kg), body mass index (BMI), smoking and stage of HIV infection according to CD4 (T helper lymphocytes).

Endothelial function study – protocol

The technique used was that described by Celermajer et al and recommended by the International Brachial Artery Reactivity Task Force¹².

The subjects fasted for at least eight hours, and were required not to exercise in the day before the test or ingest substances that might affect arterial vasodilation, such as caffeine, vitamin C and cigarette. Women should be assessed out of their menstrual period.

The subjects were placed in the supine position with the arm comfortably leaning on a proper support. The

brachial artery was imaged above the antecubital fossa in the longitudinal plane. The room was kept quiet, at a temperature of 21 degrees Celsius.

The tests were performed in a GE ultrasound scanner (VIVID 3) equipped with a vascular software for two-dimensional imaging, color and spectral Doppler, high-frequency (7-12 MHz) linear vascular transducer, and internal electrocardiogram monitor for the record of a one-lead ECG tracing.

For the assessment of endothelium-dependent vasodilation, a sphygmomanometer was placed around the patient's arm and the cuff was inflated with air up to 50 mmHg above the systolic blood pressure found. The cuff was deflated 5 minutes later, thus permitting that ischemia be followed by vasodilation via an autoregulatory mechanism and reactive hyperemia.

The brachial artery flow was continuously recorded by the color-Doppler from 30s to 90s after cuff deflation. After the reactive hyperemia phase recording, the patient rested for 10 minutes. Then, assessment of endothelium-independent vasodilation was started.

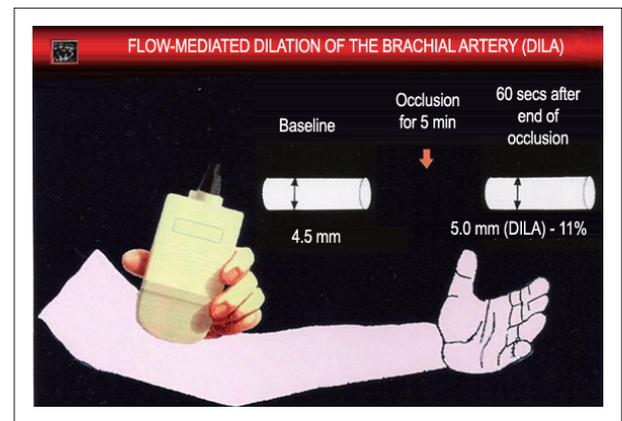


Figure 1 - Flow-mediated dilation of the brachial artery .

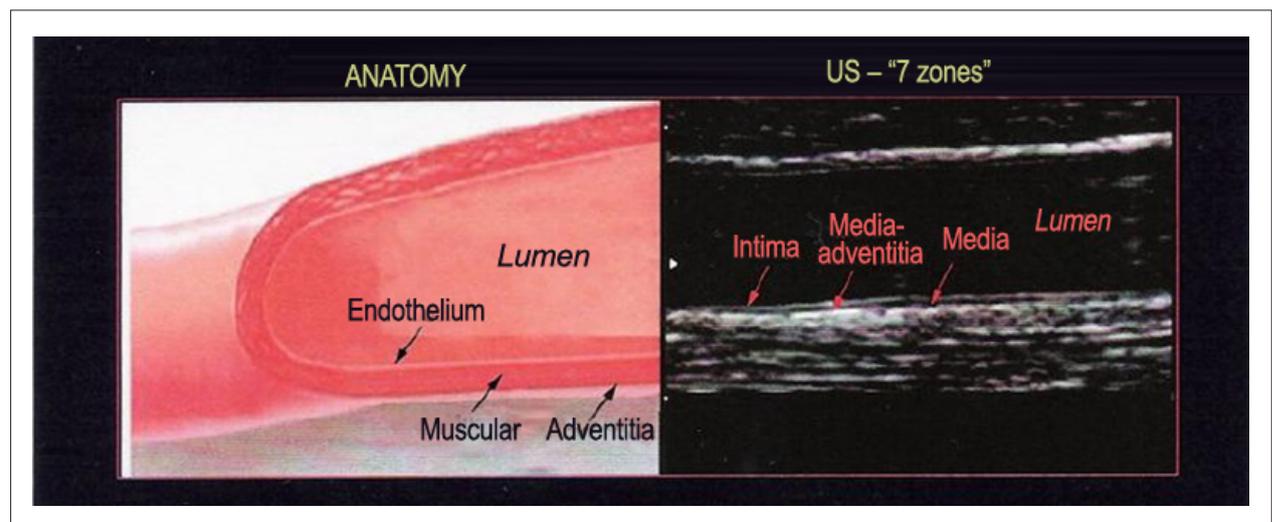


Figure 2 - Anatomy of the vessel layers on ultrasonography.

This was characterized by the administration of SL nitroglycerine spray (0.4 mg), with a 3-minute-long wait, which is the time necessary for vasodilation to occur. After this period of time, the brachial artery flow was recorded by color-Doppler. The use of nitroglycerin is contraindicated in individuals with hypotension and bradycardia.

The diameter of the brachial artery was measured from its longitudinal image, by calculating the distance between the near and far intima during diastole. Because of the wide variability in the measurement of the intima diameter, a single reference point was selected and the measurements were taken in five cardiac cycles. The measurements were taken at the same time in the cardiac cycle, using electrocardiographic gating during image acquisition. The R wave was used to identify end diastole, and the peak of the T wave, end systole.

DILA was calculated as the post-occlusion percentage increase in the diameter of the brachial artery (PODBA) in relation to baseline values.

DILA CALCULATION: $(PODBA - BDBA) / BDBA \times 100\%$

BDBA - baseline diameter of the brachial artery

Values higher than 10% of variation in brachial artery dilation in relation to baseline were considered normal.

Statistical analysis

Student's t test for independent samples or the Mann-Whitney (non-parametric) test were used to analyze the statistical significance of the differences in continuous (numeric) variables between two subgroups. The chi square test (X^2) or the Fisher's exact test were used for the comparison of categorical (qualitative) variables.

The repeated measures analysis of variance (ANOVA) was used to study the behavior of brachial artery reactivity measurements throughout time (three measurements), separated by subgroups. Bonferroni's multiple comparison test (adjusted for repeated measures) was used to identify which moments were different between themselves. One-factor repeated measures ANOVA was carried out to verify whether the behavior throughout time was different between the subgroups

The Spearman correlation coefficient was used to measure the degree of association between the relative delta and the metabolic profile; multiple linear regression analysis was used to simultaneously verify the influence of clinical and metabolic data on the relative delta of brachial reactivity to vasodilator.

Non-parametric methods were used since some variables (TG and relative deltas) were not normally distributed (Gaussian distribution) due to scattered data and/or skewness.

The significance level was set at 5%. The statistical analysis was carried out using the SAS 6.04 software (SAS Institute, Inc., Cary, North Carolina).

Ethics

This study was approved by the research ethics committee of the *Universidade Federal Fluminense* School of Medicine. For data collection, all patients gave their informed consent.

Results

Twenty seven HIV-positive patients with mean age of 41.6 years (± 8.5) from the AIDS outpatient clinic of the discipline of Infectious and Parasitic Diseases of the *Universidade Federal Fluminense* School of Medicine were selected. Of these, 15 patients (55.6%) were receiving protease inhibitors.

A group of 16 patients (37.2%) with mean age of 43.8 (± 4.3) comprised the control group.

Demographic, clinical and laboratory variables are shown in Tables 1 and 2.

No significant difference was found in relation to age and gender between the HIV-positive and negative groups ($p = 0.27$).

Continuous variables such as waist circumference (WC) and HDL-c were significantly lower in the HIV-positive group, with $p = 0.010$ and $p = 0.050$, respectively.

HIV-negative patients with hypertension (93.8% versus 14.8%; $p < 0.0001$) had higher blood pressure levels than HIV-positive patients.

TG was the predominant metabolic variable in the subgroup of HIV-positive patients receiving PI with $p = 0.004$ and $p = 0.009$ in relation to HIV-negative patients not receiving PI.

Results of brachial artery ultrasound studies are shown in Table 3.

Using the repeated measures ANOVA, brachial reactivity to reactive hyperemia and vasodilators was evaluated in HIV-positive and HIV-negative patients at three timepoints (baseline,

Table 1 - Analysis of demographic, clinical and laboratory variables according to the HIV status

Variable	HIV		p value
	positive (n = 27)	negative (n = 16)	
Age (years)	41.6 \pm 8.5	43.8 \pm 4.3	0.27
BMI (Kg/m ²)	22.2 \pm 2.5	23.4 \pm 4.4	0.33
Abdominal circumference (cm)	86.5 \pm 8.5	94.9 \pm 12.1	0.010
Blood glucose (mg/dL)	89.1 \pm 15.1	93.5 \pm 14.6	0.35
Total cholesterol (mg/dL)	204.9 \pm 48.4	196.8 \pm 45.0	0.59
HDL-c (mg/dL)	41.0 \pm 10.1	47.6 \pm 10.8	0.050
LDL-c (mg/dL)	138.8 ^a \pm 43.5	129.2 \pm 41.4	0.48
TG (mg/dL)	235.4 \pm 144.5	139.4 \pm 45.0	0.004
Reactive hyperemia (baseline)	3.49 \pm 0.67	3.30 \pm 0.58	0.35
Vasodilator (baseline)	3.62 ^a \pm 0.70	3.25 \pm 0.60	0.085

Data expressed as mean \pm standard deviation; ^a two patients lost.

Table 2 – Analysis of demographic, clinical and laboratory variables according to the use of protease inhibitors

Variable	Protease Inhibitor		p value
	with (n = 15)	without (n = 12)	
Age (years)	43.2 ± 8.2	39.6 ± 8.7	0.27
BMI (Kg/m ²)	21.7 ± 2.4	22.9 ± 2.5	0.23
Abdominal circumference (cm)	85.9 ± 8.7	87.3 ± 8.6	0.68
Blood glucose (mg/dL)	90.1 ± 16.8	87.9 ± 13.5	0.72
Total cholesterol total (mg/dL)	206.8 ± 52.6	202.4 ± 44.9	0.82
HDL-c (mg/dL)	38.6 ± 12.0	44.1 ± 6.3	0.13
LDL-c (mg/dL)	139.4 ^a ± 48.0	138.0 ^a ± 39.3	0.94
TG (mg/dL)	265.6 ± 120.6	197.7 ± 167.5	0.009
Reactive hyperemia (baseline)	3.65 ± 0.58	3.29 ± 0.73	0.16
Vasodilator (baseline)	3.79 ± 0.64	3.38 ^b ± 0.74	0.15

Data expressed as mean ± standard deviation; ^a one patient lost; ^b two patients lost.

60s, and 90s). A significant increase in brachial reactivity to reactive hyperemia was observed in the HIV-positive (p = 0.033) and HIV-negative (p = 0.014) subgroups up to 60 s. No statistical significance was found for HIV-positive patients at 90s.

The endothelium-independent analysis (use of vasodilator) showed a significant increase in brachial reactivity in both the positive and negative subgroups, with p = 0.0001 and p = 0.0001, respectively.

Also using ANOVA, the subgroups of patients receiving and not receiving PI were compared at 60s and 90s of brachial reactivity, showing a significant increase to reactive hyperemia in the subgroup with PI (p = 0.038) only at 60s, with no statistical significance for this measurement at 90s.

Using SL nitrate, a significant increase in brachial reactivity was observed in the subgroups with and without PI, with p = 0.016 and p = 0.0001, respectively.

Although a significant increase in brachial reactivity measurements (reactive hyperemia and vasodilator) was observed, especially from baseline to 60s, both in the HIV-positive and in the PI subgroups, this increase was differentiated, that is, HIV-positive or with PI subgroups showed significantly lower increase to vasodilator than the HIV-negative or without PI subgroups.

Based on the analysis of the relative delta (%) of brachial reactivity according to the HIV status and use of PI, we observed that, at 60s, both the HIV-positive and with PI groups had significantly lower relative delta to the use of vasodilator than the HIV-negative and without PI groups, with p = 0.020 and p = 0.034, respectively.

No statistical significance was found for the relative delta to reactive hyperemia in any of the subgroups.

The analysis of the relative delta of brachial reactivity according to the HIV status and use of protease inhibitor (PI) is shown in Tables 4 and 5, respectively.

Variables such as gender; body mass index (BMI); waist circumference (WC); blood glucose; total cholesterol (TC); HDL-c; LDL-c; and TG showed no statistical difference in

Table 3 - Longitudinal analysis of brachial reactivity to reactive hyperemia for the HIV and protease inhibitor subgroups

Subgroup	Reactivity to reactive hyperemia				p value ^a	significant [#] ^b
	Baseline	60 sec	90 sec			
HIV positive (n = 25)	3.48 ± 0.68	3.60 ± 0.68	3.55 ± 0.68		0.033	baseline ≠ 60"
HIV negative (n = 16)	3.30 ± 0.58	3.42 ± 0.61	3.40 ± 0.59		0.014	baseline ≠ 60" and 90"
I. Protease with (n = 14)	3.62 ± 0.59	3.78 ± 0.54	3.69 ± 0.61		0.038	baseline ≠ 60"
I. Protease without (n = 11)	3.29 ± 0.77	3.37 ± 0.80	3.36 ± 0.74		0.54	

Data expressed as mean ± standard deviation; ^a Repeated measures ANOVA; ^b Bonferroni's multiple comparisons, at 5% level.

Table 4 - Analysis of the relative delta (%) of brachial reactivity according to the HIV status

Brachial react.	Relative delta (%)	HIV	n	Mean	SE	Median	p value
Reactive hyperemia	60" - baseline	pos	27	4.8	1.5	3.1	0.68
		neg	16	3.6	1.6	2.9	
	90" - baseline	pos	25	2.6	1.7	3.2	0.53
		neg	16	3.3	1.5	3.9	
Vasodilator	60" - baseline	pos	25	8.1	2.2	5.2	0.020
		neg	16	14.8	2.5	15.1	
	90" - baseline	pos	24	9.6	2.1	6.3	0.055
		neg	16	16.6	3.2	16.9	

SE: Standard Error.

Table 5 - Analysis of the relative delta (%) of brachial reactivity according to the use of protease inhibitor

Brachial react.	Relative delta (%)	PI	n	Mean	SE	Median	p value
Reactive hyperemia	60" - baseline	with	15	5.3	1.9	3.1	0.40
		without	12	4.2	2.7	1.5	
	90" - baseline	with	14	2.4	2.2	2.0	0.62
		without	11	3.0	2.8	3.7	
Vasodilator	60" - baseline	with	15	3.8	1.5	4.2	0.034
		without	10	14.5	4.3	13.4	
	90" - baseline	with	14	4.8	1.7	5.7	0.012
		without	10	16.4	3.4	18.1	

SE: Standard Error.

relation to brachial reactivity.

Multiple linear regression analysis showed that only PI decreased the relative delta in HIV-positive patients in response to vasodilator at 60s and 90s, with $p = 0.032$ and $p = 0.010$, respectively.

Discussion

This study showed that HIV-positive patients receiving HAART, including PI, for more than two years, presented endothelial dysfunction in comparison to the group without PI and to the non-infected control group.

The main contribution of these findings in relation to previous studies is the demonstration of the impaired endothelium-independent vasodilation response in HIV-positive patients receiving PI.

Previous studies¹² have assessed endothelial function in HIV patients by means of the DILA technique, correlating it with the likelihood of development of coronary artery disease. DILA is a noninvasive, accurate and reproducible technique, albeit dependent on operational characteristics related to the ultrasound frequency and technical training.

Endothelial dysfunction to reactive hyperemia (endothelium-dependent) and to the use of nitroglycerine (endothelium-independent) was analyzed by means of the study of the brachial artery at different timepoints of dilation (60 and 90 seconds). According to a validated protocol¹², the nitric oxide (NO) peak release and artery dilation occur in this period.

Our study showed that both in the subgroup of HIV-positive patients and in that of patients using PI, there was increased brachial artery dilation to reactive hyperemia, with no difference in relation to the HIV-negative subgroup and the subgroup without PI.

Nolan et al¹³ did not find any difference in endothelial function when they compared the brachial artery flow in 24 HIV-positive patients treated with PI and 24 HIV-negative patients of the control group¹³.

Stein et al¹⁴ showed decreased brachial artery dilation in HIV-positive patients receiving PI in comparison to HIV-positive patients not receiving PI¹⁴.

Endothelium-independent vasodilation using exogenous NO is related to the smooth muscle function. In our study, only PI significantly influenced the relative delta to vasodilator at 60 and 90 seconds.

A study evaluated 800 asymptomatic HIV-negative patients with risk factors for cardiovascular disease by using exogenous nitroglycerin; it concluded that the reduced response to nitroglycerin was correlated with advanced age, increased total cholesterol, history of diabetes mellitus and smoking¹⁵.

Takase et al¹⁶ analyzed the interaction between hypertension and DM in endothelium-independent dysfunction in four groups divided into type-2 DM, hypertension, hypertension / DM, and control. The results showed that nitroglycerine-induced vasodilation was decreased in the group of patients with hypertension and diabetes in comparison to the control group ($p < 0.001$), thus showing that the interaction between DM and hypertension caused deterioration of both the vascular endothelium and the smooth muscle responsible for endothelium-independent vasodilation¹⁶.

The mechanism of endothelial dysfunction is explained by both the viral action and the use of HAART. Shankar et al demonstrated a reduction in brachial artery dilation in HIV-positive children not receiving antiretroviral drugs¹⁷. The virus acts on the endothelium by means of glycoprotein 120 (Gp120), leading to the production of adhesion molecules (ICAM-1), prothrombotic states, TNF alpha and interleukin 6 (IL-6).

Although PIs reduce endothelial nitric oxide synthase (eNOS), which leads to a decreased production of nitric oxide, reactive oxygen species (ROS) contribute to endothelial dysfunction, thus resulting in inflammation, cell damage, and endothelial cell apoptosis. PIs induce ROS formation¹⁸. Mondal et al¹⁹ demonstrated the presence of ROS in aortic endothelial cells of patients receiving zidovudine and efavirenz¹⁹.

PIs also lead to mitochondrial DNA dysfunction, thus contributing to increased oxidative stress. Cote et al showed the first case of mitochondrial dysfunction in HIV-positive patients receiving PI and presenting symptoms of hyperlactatemia²⁰.

One of the main mechanisms explaining PI-induced

endothelial dysfunction is related to protein kinase activation (MAPKs) by reactive oxygen species. PIs activate different types of MAPKs in different cells²¹.

ROS are also present in cells of the smooth muscle, which is responsible for endothelium-independent vasodilation, thus leading to endothelial dysfunction with the use of NTG in patients receiving PI. Chai et al^{22,23} demonstrated the presence of the superoxide anion in endothelial smooth muscle cells of patients treated with PI^{22,23}. Kim et al²⁴ demonstrated that viral proteins affect the proliferation of endothelial smooth muscle cells, which represents the greatest event in the formation of vascular lesion²⁴.

Preliminary studies showed that antioxidants such as ginsenoside and curcumin may reverse the endothelial dysfunction caused by PIs, thus decreasing ROS²⁵.

The major limitation of our study was the presence of hypertension in the group of volunteers, which, however, did not preclude the confirmation of our findings in relation to the endothelium-independent vascular dysfunction.

Conclusion

HIV-positive patients receiving PI present endothelium-independent dysfunction when compared to HIV-positive patients not receiving PI, as well as to the control group individuals.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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