

Endothelial Function and Cardiovascular Diseases in HIV Infected Patient

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The HIV epidemic has dramatically changed the paradigm for the development of drug therapy in the last 15 years. The goal is now not only to provide an effective reduction of plasma viremia, but also to reconstitute the immune deficiency due to the progression of the disease. Significant problems with the metabolism of sugars and lipids lead to the appearance of well-documented disorders such as insulin resistance, abnormalities in lipid metabolism and lipodystrophy in those patients on prolonged therapy with antiretrovirals. The question of whether or not HAART-associated lipid disorders contribute to the premature development of coronary artery disease is of major importance for the HIV community. Endothelial injury is associated with disease-related biochemical abnormalities that are implicated in HIV pathogenesis. The exploration of endothelial function began in the early 1980s at the start of the epidemic. The study of endothelial function in HIV infection and its modifications by HAART is an exciting new field in clinical research; in this review the available information on cardiovascular diseases associated with HIV infection and its treatment are discussed.

Key Words: Human immunodeficiency virus, endothelial dysfunction, endothelial adhesion molecules, antiretroviral therapy, cardiovascular risk factors, atherosclerosis, coronary heart disease.

HIV epidemic has dramatic changed the paradigm for the development of drug therapy in the last 15 years [1]. Goals are not only to provide an efficacious reduction on plasma viremia, but also attempt to reconstitution of the immunodeficiency following the progression of HIV infection [2,3]. Potent enzyme inhibitors of the HIV life's cycle became available in the decade of 90's [4]. Several trials and cohort of patients receiving the combination of such inhibitors had documented a dramatic changed in the natural course of HIV infection [5-8].

Nevertheless, in the early 2000's the anticipated adverse reaction of the antiretroviral following prolonged required continuously and in interrupt of its uses, started to challenge the goals of the, highly active antiretroviral therapy (HAART) [3,9,10].

Serious interaction with the metabolism of sugar and lipids lead to the appearance of well-documented disorders such as insulin resistance, abnormalities in lipid metabolism and lipodystrophy, particularly associated with protease-inhibitors [11-18]. Consequently premature coronary diseases and other cardiovascular disorder have been documented associated to the use of ARV drugs [19-21]. Despite of documentation of myocardial infarct and high rate of atherosclerosis and cardiovascular diseases in HIV infected patients associated with HAART prospective observational study reported the incidence of myocardial infarction increased, relative risk was 1.26 (95% CI 1.12 to 1.41), directly with longer exposure with antiretroviral therapy in HIV-infected patients [22]. However, studies have shown conflicting data regarding myocardial

infarct [23-26]. It is debating the true mechanism of link between HIV, use of ARV and clinical outcome [2,27-29]. One possible mechanism it has been proposed is the cholesteryl ester accumulation in macrophages independent of dyslipidaemia. In murine model protease inhibitors are documented to up regulating CD-36 leading to the induction of atherosclerosis [30]. Others proposed mechanism are related to PIs inhibition of human proteins that are involved in lipid metabolism, including aspartyl proteases, which have homology to the catalytic site of HIV-1 protease, to which all PIs bind. One such protein is the LDL receptor-related protein (LRP), suggesting that PI-associated lipid abnormalities may be partly accounted for by reduced clearance of LRP ligands [31,32]. On the other hand, in the mouse, conditional liver-specific disruption of LRP is only associated with hyperlipidaemia after equal disruption of the LDL receptor, suggesting that LDL receptor fully compensates for the lack of LRP in these animals. Moreover, clearance of triglycerides-rich particles does not seem to be impaired in PI-treated mice [33,34].

Also it possible that, strong activation in the liver of lipogenic genes that are under the control of sterol regulatory element-binding protein (SREBP)-1c also termed as ADD1 - adipocyte determination and differentiation factor 1 [35,36]. In these particular experiments, the abundance of SREBP-1c protein in the nucleus of liver cells was increased in ritonavir-treated animals [36]. This mechanism may also account for retarded degradation of apolipoprotein B-100 within the liver cells, and may thus indirectly contribute to overproduction of VLDL particles [37,38]. Microorganism are studied for their role in inducing an inflammatory response in endothelial cells (such as *Chlamydia pneumoniae*, cytomegalovirus, herpes simplex virus and Epstein-Barr virus), and recently HIV was documented to interact with endothelial cells membrane and to initiate inflammatory and biochemical intracellular reactions [39]. Atherosclerosis is a consequence of infection-triggered endothelial damage [40].

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The question of whether or not HAART-associated lipid disorders contribute to the premature development of coronary artery disease is of major importance for the HIV community and this concern has been substantiated by a series of reports on PI-treated HIV-infected patients who experienced unexplained cardiovascular events [41-46]. Even though HIV infection and HAART are associated with dyslipidaemia, current data do not indicate that PIs are independently associated with an increased risk for cardiovascular morbidity and mortality [24,47].

Endothelial Cell Function and HIV

It is well established that predisposing to thrombosis, leukocyte adhesion, and smooth muscle cell proliferation plays a pivotal role in the development, progression, and clinical manifestations of atherosclerosis [39,40,48-50]. Endothelium is involved in important homeostatic mechanisms of non-thrombotic vascular surfaces, vascular tone regulation and immunomodulation [39,51]. Several different clinical conditions, such as, hypertension, dyslipidaemia, diabetes mellitus, contribute to endothelial dysfunction thought to be a major link between infection, inflammation and atherosclerosis [39,51-55].

The HIV epidemic introduces a new agent that has been associated with endothelial dysfunction [56,57]. Several observations in pathophysiologic studies in humans and animals led to the formulation of the response-to-injury hypothesis of atherosclerosis, which initially proposed that endothelial denudation was the first step in atherosclerosis [52].

Injured endothelium lead to localized inflammatory response of which the direct consequence is the occurrence of occlusive thrombotic events mediated by leukocyte recruitment, platelet adhesion and aggregation, blood clotting activation and fibrinolysis derangement [40,51]. Endothelial injury is associated with disease-related biochemical abnormalities that are implicated in HIV pathogenesis. For instance, entry of virus into endothelial cells could possibly occur through CD₄ antigen, galactosyl-ceramide receptors [40,51,58-60], or chemokine receptors [61-63]. Endothelial activation may also occur either by cytokines secreted in response to mononuclear or adventitial cell activation by HIV virus or by the effects of gp120 and Tat, both secreted HIV-associated proteins, on endothelium [64,65].

Intercellular adhesion molecule (ICAM-1) and P-selectin are documented factors implicated in myocardial infarction and atherosclerosis [52]. High levels of these adhesion molecules represent early markers of the development of atherosclerosis [66,67]. Of interest, beside soluble adhesion molecules, other factors such as fibrinolytic factors, tissue plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1) have also been considered to be markers of endothelial dysfunction [68-70].

Endothelial function begins to be explored in HIV infected patients in the early 1980s since HIV epidemic started [71-73]. Increased levels of soluble adhesion molecules was documented in different stages of HIV [66,67,74,75].

HIV protease inhibitors have been successfully used against HIV infection, many metabolic side effects and premature cardiovascular diseases are often associated with this therapy [13,76-78]. In experimental model human endothelial cells treated with ritonavir showed a significant decrease in cell viability and an increase in cytotoxicity in a time- and dose-dependent fashion. Mitochondrial DNA was also substantially damaged with ritonavir treatment by long polymerase chain reaction analysis. HIV protease inhibitor ritonavir at concentrations near clinical plasma levels is able to directly cause endothelial mitochondrial DNA damage and cell death mainly through necrosis pathways but not through apoptosis [79]. Other observations with protease inhibitors alone on vascular function, was the administration of the protease inhibitor, indinavir, resulting in impaired endothelium function by measure of leg blood flow responses to intra-arterial infusions of methacholine chloride, sodium nitroprusside and N^G-mono-methyl-L-arginine (L-NMMA). After 4 weeks of daily oral indinavir, they found that in the absence of HIV-infection, indinavir causes vascular dysfunction most likely at the level of endothelial nitric oxide production [80], and increased carotid intima thickness or atherosclerotic lesions [81].

Available Methods for Assessment of Endothelial Function

Endothelial function assessment could be performed either invasively or non invasively [82-85]. Non-invasive models include study of biomarkers that are present on the surface of endothelial cells or are expressed in response to several stimuli and have an important role in the process of leukocyte rolling, firm adhesion and transendothelial migration, biomarkers are either present on the surface of endothelial cells or are expressed in response to several stimuli and have an important role in the process of leukocyte rolling, firm adhesion and transendothelial migration [52,66,67]. Soluble CAMs are considered reliable biomarkers of atherosclerosis development and severity and to add to the predictive value of classic risk factors for coronary artery disease in healthy individuals and in patients [86]. Another non-invasive technique is the use of ultrasonography to assess the degree of flow-mediated dilatation (FMD) of the brachial artery following an ischaemic stimulus [25,87]. Endothelial function at the brachial artery provides a surrogate measure of the coronary circulation and a correlate of the severity of coronary artery disease [25,87,88]. Accordingly, abnormal brachial artery endothelial function has been associated with a wide spectrum of cardiovascular risk factors, including dyslipidaemia, smoking, diabetes and hypertension [88-91]. Endothelial dysfunction is considered the key step in the development of atherosclerosis and it is

known to be an early predictor of future cardiovascular events in patients without and with known cardiovascular disease [92-95].

Another approach to study endothelial function is to assess invasively, by studying blood flow responses, either intracoronary or intra-arterial, to infusions of metacholine chloride, sodium nitroprusside and N^G-mono-methyl-L-arginine (L-NMMA) [91].

Clinical Assessment of Endothelium in HIV Infection

Alternatively to the invasive measurement of endothelial function derived from direct infusions of the above mentioned agents into the arteries, one can use a less invasive method of indirect assessment using the technique of post ischemic flow-mediated dilation.

Stein et al. reported a cross sectional study involving 37 HIV infected patients on antiretroviral therapy (22 subjects were on protease inhibitors containing regimen and 15 were on PI-sparing regimen). They found that endothelial function was impaired in HIV infected subjects on PI-containing regimen and HIV infected patients with no ARV use had a normal FMD and they concluded that protease inhibitors are associated with atherogenic lipoprotein changes and with endothelial dysfunction [98]. Two recent studies on the effect of statins on endothelial functions and lipid profile in HIV infected patients receiving protease inhibitors, showed that pravastatin reduced concentrations of atherogenic lipoproteins and that endothelial function tended to increase in patients receiving pravastatin; however, the difference between treatment regimen was not statically significant [98-100].

In order to determine the impact of HIV infection and antiretroviral therapy on markers of early vascular disease in children, Charakida et al. [101] studied 83 HIV-infected children (56 had taken HAART, of whom 31 received a regimen containing protease inhibitors; 27 were never treated and a control group of 59 healthy children). Carotid intima-media thickness (IMT) and endothelial function were measured by brachial artery flow-mediated dilatation (FMD). Children exposed to protease inhibitors had greater IMT compared with both non-PI-treated children and untreated children ($p=0.02$). FMD was also significantly reduced in HIV-infected children compared with control subjects ($p=0.02$). Comparisons of different treatment exposure groups revealed that endothelial function was impaired by a mean of 3.6% (95% CI, 1.8 to 5.3; $p<0.001$) for children exposed to PIs compared with untreated children and by a mean of 1.8% (95% CI, 0.01 to 3.5; $p=0.05$) compared with non-PI-treated patients. This study demonstrates that structural and functional changes of the vasculature are already present during childhood in HIV-infected children and these changes were most pronounced in children receiving protease inhibitors, but were also observed in non-protease inhibitors treated and untreated

children and the findings of the study support a role for both HIV infection itself and ART, particularly protease inhibitors, in the pathogenesis of early vascular disease, likely to be relevant to future clinical atherosclerosis [101]. Stein et al., recently, presented preliminary data from a prospective, randomized, multicenter substudy (A5152s) to evaluate the effects of antiretroviral therapy on endothelial function in treatment-naïve HIV infected individuals. Patients in the parent study, A5142, were randomly assigned to one of three antiretroviral regimens: efavirenz + 2 NRTI (PI sparing regimen); lopinavir/ritonavir + 2 NRTI (NNRTI sparing regimen); and lopinavir/ritonavir + efavirenz (NRTI sparing regimen). Endothelial function determined by flow-mediated vasodilatation was measured at baseline, week 4 and at week 24. Subjects included 82 HIV infected treatment naïve patients with a median age of 34 years. Prior to starting antiretroviral therapy, FMD was impaired. After 4 weeks of treatment, FMD had significantly improved by 1.1% ($p=0.003$) and the improvement was of similar magnitude in each arm. After 24 weeks of treatment, FMD had increased significantly by 1.9% ($p<0.001$) and it was of similar magnitude for each arm. This suggests that the use of three different antiretroviral regimens rapidly improved endothelial function in treatment naïve patients with HIV infections and that the benefits were similar regardless of antiretroviral regimen and they appeared as early as after 4 weeks and persisted at 24 weeks of treatment and they concluded that antiretroviral treatment in treatment-naïve patients improved vascular reactivity and it may decrease short term cardiovascular risks [56].

Conclusions

Endothelial dysfunction is associated with diabetes mellitus, hypertension, dyslipidemia, tobacco use and other metabolic disorders and is a predictor of future cardiovascular events. Endothelial dysfunction has also been associated with HIV infection and HIV therapy.

Protease inhibitors, a main component of antiretroviral therapy, induce many deleterious metabolic effects, such as dyslipidemia, insulin resistance and other metabolic disorders and may expose HIV infected patients to an increase risk for coronary artery disease. Antiretroviral therapy may cause endothelial dysfunction by a direct effect on the endothelial cells or by indirect mechanisms, possibly in synergy with the HIV virus on endothelial cells, or through its effects upon the lipid and glucose metabolism. However, there are still conflicting results regarding the effects of HIV infection and its therapy on endothelial dysfunction, as assessed by brachial artery flow mediated vasodilatation, some showing a worsening and others showing improvement of endothelial function (Cotter B. Endothelial dysfunction in HIV infection, In press).

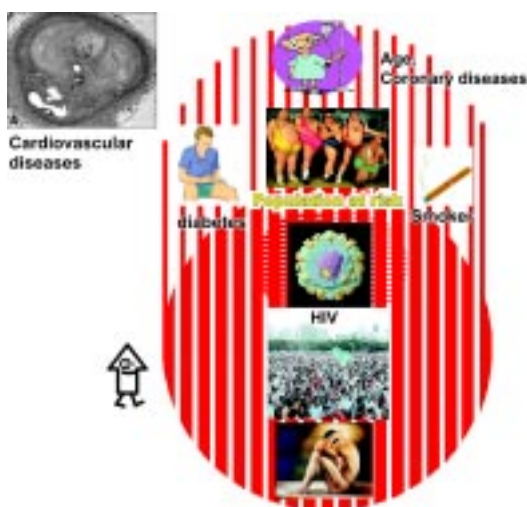
The introduction of highly active antiretroviral therapy has significantly improved the prognosis of HIV infected

patients with a dramatic reduction in both morbidity and mortality, with an improvement in patients' quality of life. However, in recent years, several clinical studies have hinted at an increased risk for cardiovascular disease (CAD), particularly among patients receiving protease inhibitors. Although the DAD Study Group found that the relative risk of cardiovascular disease increases with the duration of antiretroviral therapy, the absolute risk for cardiovascular disease remain low for most patients, except those with multiple traditional risk factors for coronary artery disease, and is far outweighed by the benefits of antiretroviral therapy in terms of reduced risks of AIDS and death in most HIV infected patients.

Traditional cardiovascular risk factors for CAD, including diabetes mellitus, hypertension, dyslipidemia, tobacco use, sedentary life style, obesity and family history need to be assessed first in HIV infected patients. In the near future, it may be possible to draw a schematic (Figure 1) showing the distribution among the general population, in levels of being either close to or far from an undesirable cardiovascular event, based on traditional risk factors for CAD; however, at this time there are no conclusive data to support HIV infection and its therapy to be considered as a definite risk factor for coronary artery disease.

Antiretroviral therapies have been considered among the miracle drugs of recent decades with a significant reduction in both mortality and morbidity and improvement in quality of life for HIV infected patients and there must be unequivocal evidence that drastic changes in antiretroviral therapy are warranted. So far, no conclusive data regarding risks for cardiovascular events support drastic changes in antiretroviral therapy. Physicians taking care of HIV infected patients should first emphasize the need for modifications of the traditional risk factors for CAD, such as smoking cessation, diet and exercise and the need to treat hypertension and other metabolic disorders, before considering changes in antiretroviral therapy.

Figure 1. Hypothetical diagram of natural history of cardiovascular diseases



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