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ORIGINAL ARTICLE

Overexpression of Endothelin-1 Leads to More Severe Pulmonary Complex Vascular Lesions Associated with the Human Immunodeficiency Virus

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Background and Aims. Despite increase in survival of HIV patients due to highly active antiretroviral therapy (HAART), non-infectious complications are still prevalent such as presentation of lung vasculopathy, even in asymptomatic patients. Endothelin-1 (ET-1) is a potent vasoconstrictor that causes pulmonary vasculopathy. Participation of this protein in the pulmonary circulation in HIV patients has not been elucidated. In this work we studied the presence and expression of ET-1 in pulmonary complex vascular lesions associated with human immunodeficiency virus (PCVL/HIV).

Methods. We used immunohistochemistry and immunochemiluminescence (imagej) to determine the different degrees of expression of ET-1 in PCVL/HIV in comparison with non-PCVL/HIV. Reagents used were anti-endothelin-1 and an automated system. All data are presented as mean and standard deviation (SD). Differences were analyzed with one-way ANOVA; $p < 0.05$ was accepted as statistically significant.

Results. Lung tissues from 56 patients who died from complications of HIV pulmonary infection and with PCVL were studied. Histological evidence of pulmonary vasculopathy was shown as different types (proliferative, obliterative and plexiform). A statistically significant increase in ET-1 expression was observed in all PCVL/HIV tissue samples and is associated directly with different grades of severity of endothelial dysfunction.

Conclusions. ET-1 has a relevant role in the pathogenesis of pulmonary vasculopathy in acquired immunodeficiency syndrome (AIDS) patients. It is necessary to determine in the future the participation of ET-1 and other mechanisms involved in PCVL/HIV.

Key Words: Endothelin, Human immunodeficiency virus, Pulmonary circulation.

Introduction

The epidemic of human immunodeficiency virus (HIV) currently affects >34 million people globally, with a mortality rate of 1.7 million per year (1). Many of these deaths in developing countries were due to noninfectious complications like cardiovascular diseases (2). HIV infection is a risk factor for the development of pulmonary arterial hypertension (PAH), which is seen with a 2500-fold higher increase in HIV-infected vs. the uninfected population (3). PAH is characterized by the formation of pulmonary complex vascular lesions (PCVL). The pathogenesis for the development of PCVL associated with infection with human immunodeficiency virus (PCVL/HIV) is still unclear (4).

Endothelin-1 (ET-1) is a 21 amino acid peptide released from endothelial cells under hypoxic conditions that can bind to two receptors: endothelin receptor A (ET_A) expressed only by smooth muscle cells (SMC) or endothelin

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receptor B (ET_B) expressed by both endothelial and SMC. Binding of ET-1 to ET_A induces calcium influx, contraction and proliferation of SMC. The participation of transcription factor c-fos has also been studied (5).

Endothelin-1 (ET-1), a potent vasoconstrictor, plays a role in the pathogenesis of pulmonary vasculopathy (6). In addition to its vasoconstrictive properties, ET-1 also stimulates DNA synthesis and proliferation in pulmonary arterial smooth muscle cell (PASMCs) (7).

HIV has never been shown to directly infect pulmonary vascular endothelial cells. However, HIV viral antigens are present in pulmonary endothelium and may directly stimulate abnormal apoptosis, growth and proliferation. Excessive production of ET-1 has been implicated in endothelial cell dysfunction and in pathogenesis of several causes of PAH not demonstrated in pulmonary tissues of HIV patients (8). In the setting of HIV infection, HIV-related proteins may affect ET-1 production, not only by endothelial cells but also by inflammatory cells. Glycoprotein 120 (gp120), a viral protein necessary for the binding and entry of HIV into macrophages, has been shown to target human lung endothelial cells, increase markers of apoptosis, and stimulate the secretion of ET-1 (9). The HIV-1 Tat protein interacts with at least three different types of receptors present on the surface of endothelial cells, which trigger various biological responses in the endothelium and may therefore be involved in the pathological processes that occur in acquired immunodeficiency syndrome (AIDS) including PAH (10).

Endothelin-1 (ET-1) is a potent vasoconstrictor linked to many effects in different pathologies but mainly in pulmonary circulation (11). This protein in lung vascular disease has been widely studied, but with regard to HIV there is limited information.

Demonstration of ET-1 participation in lung endothelial lesions associated with HIV will allow modification of the treatment caused due to endothelin receptor antagonist (ERA) drugs in the therapeutic field (12).

In this work we studied the presence and expression of ET-1 in non-PCVL/HIV and PCVL/HIV histological tissues.

Materials and Methods

Tissue and Histological Examination

Pulmonary tissues from patients who died from complications of HIV pulmonary infection and with PCVL were collected during autopsies. For this study we used a simplified Heath and Edwards classification (13) (Table 1).

Table 1. Heath and Edwards pulmonary circulation simplified pathology classification

Grade I. Hypertrophy of the media of small arteries and arterioles and proliferation of the intima
Grade II. Thickening of the middle layer with hypertrophy and hyperplasia, showing plexiform lesions in the muscle
Grade III. Injury and cavernous angioma, with intimal hyalinization, fibrosis and/or necrotizing artery

Immunohistochemistry and Immunochemiluminescence

Reagents used were anti-endothelin-1 (ab49591, Abcam, Cambridge, MA). For immunohistochemistry we used the automated system from Ventana (Tucson, AZ). ET-1 quantification was measured by chemiluminescence on slides stained by immunohistochemistry and processed with the image processing software *ImageJ* provided by the U.S. National Institutes of Health. The expression of the protein was measured in pixels (arbitrary units).

Ethics

This work was approved by the internal Ethics and Research Committee of the National Institute of Respiratory Diseases "Ismael Cosío Villegas" (number: B-19-13).

Statistics

All data are presented as mean and standard deviation (SD). The differences were analyzed with one-way ANOVA; $p < 0.05$ was accepted as statistically significant. Statistical Package for the Social Sciences (SPSS® v.20) was used.

Table 2. Summary of demographic and disease characteristics of the HIV/AIDS study group

N	56
Mean age, years (range)	38.8 ± 2.82 (22–60)
Male (%)	85
HIV risk factor (%)	
Intravenous drug use	1
Homosexual	40
Heterosexual	15
Pulmonary coinfections	
PCP	20
Polymicrobial	16
CMV	10
Histoplasma sp.	6
MTB	4
Median CD4, SD cell count, cells/μl (range)	37 ± 5 cells (1–115)
Viral load count, SD, (range)	350,000 ± 150,000 (150,000–1 million)
Median follow-up, years (range)	3.0 (0–9)
HAART	
37 HAART used before hospitalization	
10 HAART initiated at or after HIV diagnosis in hospital	
9 Never received HAART	

PCP, *Pneumocystis jiroveci*; CMV, cytomegalovirus; MTB, mycobacterium tuberculosis; HAART, highly active antiretroviral therapy.