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Original article

Effect of antiretroviral therapy in thromboregulation through the hydrolysis of adenine nucleotides in platelets of HIV patients

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ABSTRACT

The human immunodeficiency virus (HIV) infection results in biochemical and vascular dysfunctions. The highly active antiretroviral therapy (HAART) markedly reduces mortality and opportunistic diseases associated with acquired immunodeficiency syndrome (AIDS). This increased survival time predisposes the development of cardiovascular diseases. Platelets present purinergic system ectoenzymes such as E-NTPDase, E-5′-nucleotidase and E-ADA on its surface. In view of this, the aim of this study was to evaluate the activity of these ectoenzymes in platelets as well as the platelet aggregation and lipid profile of patients with HIV infection and also patients receiving HAART. The results showed an increase in the E-NTPDase activity for ATP hydrolysis in the HIV group compared with the control group and the HIV/HAART group. When assessing the activity E-NTPDase hydrolysis to ADP, the results revealed an increase in activity in the HIV group when compared to the control group, and a decrease in activity when in the HIV/HAART group when compared to the control and HIV groups. The activity of E-5′-nucleotidase revealed an increase in AMP hydrolysis in the HIV group, as the results from control and HIV/HAART groups showed no statistical difference. Regarding the E-ADA activity, the HIV and HIV/HAART groups revealed a decreased deamination of adenosine when compared with the control group. Furthermore, we observed an increased platelet aggregation of HIV/HAART group compared with the control group. Thus, our results suggest that antiretroviral treatment against HIV has a significant effect on the activity of purinergic system ectoenzymes demonstrating that thromboregulation is involved in the process.

1. Introduction

The acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV), is a pandemic disorder, representing a major health issue today, due to its severity, transmission and global prevalence. The active antiretroviral therapy (HAART) stops the viral replication and prevents disease progression, turning the HIV infection into a chronic condition. However, despite its many benefits, HAART may lead to a series of physiological and biochemical disorders [1], such kidney, liver and cardiovascular disorders and cancer [1,2]. Cardiovascular disease has emerged as a significant cause of mortality in treated and non-treated HIV-infected individuals [3]. Cardiovascular complications in HIV-infected individuals include thrombotic microangiopathy, arterial disease, dilated cardiomyopathy, abnormal coronary artery pathology, and myocarditis [3–6]. Macrovascular and microvascular disorders involve complications such as thrombosis and atherosclerosis which can be accelerated by platelet activation [7,8]. The increased platelet aggregation and release of vasoactive mediators are contributing factors to the occlusion of the blood vessel function [9].

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Platelets are important mediators in the maintenance of endothelial integrity and hemostasis and contribute to other fundamental biological processes, including inflammation and defense against pathogens [10,11]. Tsegaye et al. [12] suggest that platelets might constitute a so far unappreciated innate defense against HIV-1 transmission and spread since activation of platelets induces the release of the chemokine CXCL4 which suppresses this infection at the viral entry stage. However, how platelets are activated in the context of HIV infection, as well as the consequences of activation for platelet function and HIV interactions, are largely unknown. Since the secretion by platelets of purinergic products, ATP and ADP, is considered of major importance for the progression of platelet activation we hypothesized that these molecules could be involved in platelet response to HIV infection. ADP plays a key role in platelet activation and recruitment to the blood vessel wall, while adenosine and high concentration of ATP inhibit ADP-induced platelet aggregation. In fact, the ATP-gated channel P2X1 and the two G protein-coupled P2Y1 and P2Y12 ADP receptors selectively contribute to platelet aggregation being considered as potential targets for antithrombotic drugs [13,14]. To control the extracellular concentration of these signaling molecules in physiological levels there is a cascade of ecto-enzymes on surface of platelets [15]. Alterations in the activity of these platelet enzymes have been observed in many toxicological and pathological conditions such as rheumatoid arthritis, multiple sclerosis, diabetes mellitus, neoplasias, Chagas Disease and ischemic heart disease [16–23].

Therefore, taking into account the importance of ectoenzymes in the maintenance of hemostasis and the presence of cardiovascular complications in HIV patients, this study aimed to evaluate the E-NTPDase, E-5′-nucleotidase and E-ADA activities in platelets as well as the platelet aggregation and lipid profile of HAART-treated and non-treated HIV-infected patients, in order to verify whether the platelet purinergic signaling is involved in the response to HIV infection and its treatment.

2. Material and methods

2.1. Chemicals

Nucleotides, adenosine, bovine serum albumin, Trizma base, HEPES and Coomassie Brilliant Blue G were obtained from Sigma-Aldrich (St. Louis, MO, USA). All the other chemicals used in this experiment were of analytical grade and of highest purity.

2.2. Patients and samples

The sample consisted of sixty patients with HIV infection from the Hospital of the Federal university of Santa Maria (Santa Maria, RS, Brazil). The subjects were divided into three groups: control (healthy volunteers with serology nonreactive for HIV), HIV (HIV-infected patients) and HIV/HAART (patients in treatment with HAART). The HIV group consisted of 10 patients who were not taking antiretroviral therapy, these patients never used HAART and with an average age of 29.53 ± 1.41. The HIV/HAART group was constituted of 50 patients in treatment with antiretroviral therapy and with an average age of 35.68 ± 1.41. The control group (control) was constituted of 20 healthy volunteers with an average age of 27.93 ± 1.48 years old and serology nonreactive for HIV. All participants presented normal blood pressure and were free from diabetes mellitus, alcoholism and cigarette smoking. The average use of antiretroviral drugs was 7 years which characterizes a chronic drug use. All subjects gave written informed consent to participate in the study. The Human Ethics Committee of the Health Science Center, from Federal University of Santa Maria approved the protocol under number 08163912.6.0000.5346.

2.3. Platelet preparation

The platelet-rich plasma preparation (PRP) was prepared by the method of Pilla et al. [24] modified by Lunkes et al. [20]. Briefly, peripheral blood was collected in 0.129 M sodium citrate as anticoagulant and centrifuged at 160*g* for 15 min. Afterwards, the PRP was centrifuged at 1400*g* for 30 min and washed twice with 3.5 mM HEPES buffer, pH 7.0, containing 142 mM NaCl, 2.5 mM KCl and 5.5 mM glucose. The washed platelets were resuspended in HEPES isosmolar buffer.

2.4. Cellular integrity

The integrity of the platelet preparation was confirmed by determining the lactate dehydrogenase (LDH) activity in intact and disrupted platelets using the kinetic method of the Labquest apparatus (Diagnostics Gold Analyzer). The procedure was repeated before and after the incubation period. The protocol was carried out according to the manufacturer's instructions. Triton X-100 (1%, final concentration) was used to disrupt the platelet preparation. The enzymatic activity is expressed as units per liter, and one unit (1U) corresponds to 1 μmol of NADH formed per minute per liter.

2.5. Coagulation parameters

Total blood was collected in tubes containing 7.2 mg dipotassium EDTA as anticoagulant and the quantitative determination of platelets was performed by automated hematology analyzer (SYSMEX XT-1800i, Roche Diagnostic, USA) and in tubes containing 0.129 M sodium citrate to prothrombin time (PT) and partial thromboplastin time (PTT) measurement by an automated coagulation analyzer (SYSMEX CA-1500, Roche Diagnostic, USA). PT and PTT are expressed in seconds and quantitative determinations of platelets are expressed as quantity per microliter.

2.6. Protein determination

After the platelets separation, the protein was adjusted to 0.4–0.6 mg/mL for enzymatic assays according to Bradford [25] using bovine serum albumin as standard.

2.7. E-NTPDase and E-5′-nucleotidase activities

Ectonucleotidase activities were determined using a PRP preparation according to Pilla et al. [24]. Briefly, to determine E-NTPDase activity, 20 μL of PRP preparation was added to the system mixture, which contained 5 mM $CaCl_2$, 100 mM NaCl, 4 mM KCl, 5 mM glucose and 50 mM tris-HCl buffer, pH 7.4. The reaction was started by the addition of 20 μL of ATP or ADP (1 mM) as substrate. For AMP hydrolysis, E-5′-nucleotidase activity was determined as described above, except that 5 mM $CaCl_2$ was replaced by 10 mM $MgCl_2$ and the nucleotide added was 2 mM AMP. Both reactions were stopped by the addition of 200 μL of 10% trichloroacetic acid (TCA) to provide a final concentration of 5%. After this, the inorganic phosphate released by ATP, ADP and AMP hydrolysis was determined in triplicate by the Chan et al. method [36] using KH_2PO_4 as standard. The same process was carried out in control tubes to exclude non-enzymatic hydrolysis, by adding 20 μL of protein to the reaction medium after TCA. Results were expressed as nmol inorganic phosphate released/minute/milligram of protein (nmol Pi/min/mg protein).