INCREASED LEUKOCYTE / PLATELET INTERPLAY WITH THE ENDOTHELIUM IN ABACAVIR-TREATED HIV PATIENTS

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BACKGROUND

Abacavir (ABC) has been associated with a risk of myocardial infarction (1). We have demonstrated in *in vitro* experiments that clinical concentrations of ABC, but not of tenofovir disopropil fumarate (TDF), have both **pro-inflammatory** (inducing leukocyte-endothelium interactions) and **pro-thrombotic** (causing the interplay of platelets with endothelial cells or leukocytes) **actions** (2-5). Furthermore, ABC promotes thrombus formation in a well-established *in vivo* model (6).

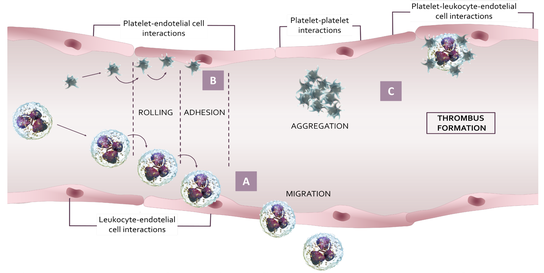

Figure 1. Process of vascular inflammation and thrombi formation induced by ABC. ABC induces the recruitment of leukocytes by the endothelium **(A)**, one of the earliest features of inflammation-associated cardiovascular disease. Moreover, ABC promotes the adherence of platelets to endothelial cells **(B)**, a process that could be implicated in the formation of thrombi **(C)**.

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OBJECTIVE

The aim of this study was to analyze the interactions of leukocytes and platelets isolated from blood of **HIV patients treated with ABC** with a non-infected endothelium, and to compare them with those of patients treated with TDF.

METHODS

This is a non-random prospective observational study in which we employed blood cells from HIV patients at Hospital Clínico Universitario de Valencia who had been receiving treatment for at least 6 months with a cART regime that included either ABC (n=27) or TDF (n=33).

Glass coverslips containing monolayers of confluent human umbilical vein endothelial cells (HUVEC) from healthy donors were inserted into a flow chamber for adhesion assays. Human leukocytes or platelets (isolated from blood of HIV patients), were drawn across the HUVEC monolayer (shear rate=0,7 dynes/cm²). Platelets were previously labelled with Alexa-Fluor®488-CD41 (platelet marker) in order to be visualized.

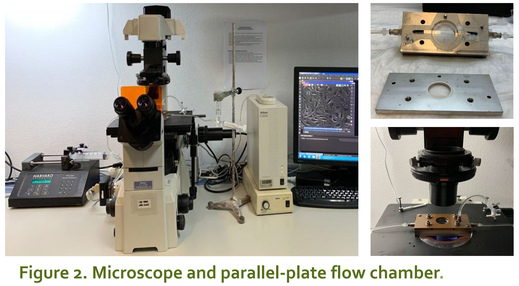

Figure 2. Microscope and parallel-plate flow chamber.

Images were recorded by an inverted microscope equipped with an Epi-Fluorescence system.

To measure **leukocyte-endothelium interactions**, rolling flux was calculated by counting the number of cells rolling across 100 µm² of monolayer during a 1-min period. Adhesion was determined after 5 min of perfusion by analysing 5-10 high power (40x) fields. Leukocytes were considered to be adherent after 30 s of stable contact with the monolayer.

The adhesion of **platelets** to the endothelium was determined by fluorescence analysis of 5-10 high power (40x) fields after 5 min of platelet perfusion and subsequent washing.

Statistical analysis: Data were compared using an unpaired t test with Welch's correction. Data were mean ± S.E.M.

RESULTS

60 patients were included in the study, 27 on ABC and 33 on TDF. There were no significant differences in demographic and cardiovascular risk parameters between the two groups. Patients on ABC were taking: 1) Triumeq, or 2) Kivexa with Insentress or Reyataz. Patients on TDF were taking: 1) Genvoya or 2) Symtuza or 3) Eviplera or 4) Descovy with Rezolsta or Tivicay or Isentress or 5) Truvada with Tivicay or Isentress.

Table 1. Characteristics and antiretroviral treatments of the patients. Data are expressed as mean ± S.E.M or as percentage of total.

	Tenofovir (TDF)	Abacavir (ABC)
Number of subjects	33	27
non-HIV medical history		
Age (years)	38,94 ± 1,59	42,77 ± 1,63
Sex (Men %)	27 (87,10%)	21 (77,78%)
Body mass index (kg/m2)	25,20 ± 0,68	26,06 ± 0,70
Current smokers	10 (32,26%)	4 (14,81%)
HIV medical history		
Current antiretroviral therapy duration (months)	46,87 ± 6,44	49,09 ± 8,53
CD4+ T cell count (cells/mm³)	761,1 ± 63,75	860,7 ± 58,26
Transmission route (%)		
HTSX	6 (18,18%)	9 (33,33%)
HO/BI	26 (78,79%)	16 (59,26%)
UDVP	0	2 (7,41%)
HEMO	1 (3,03%)	0

LEUKOCYTE-ENDOTHELIUM INTERACCIONS WERE HIGHER IN ABC-GROUP THAN IN TDF-GROUP

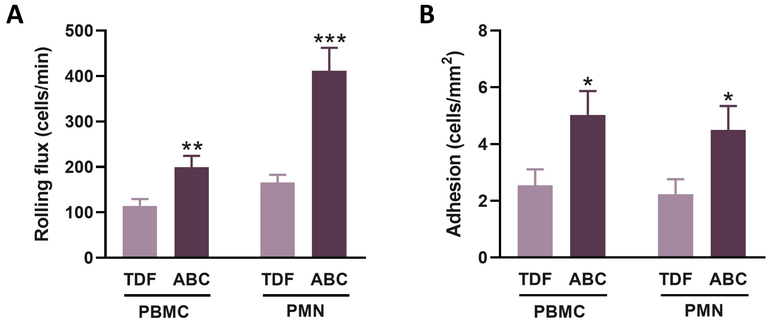

Figure 3. Leukocyte-endothelium interactions in HIV-infected patients. Peripheral blood mononuclear cells (PBMC) and polymorphonuclear cell (PMN) interactions with the endothelium were evaluated with a parallel-plate flow chamber system. Suspensions of leukocytes from TDF-treated and ABC-treated HIV patients were drawn across a non-HIV human endothelial monolayer. Images were recorded using an inverted microscope (Nikon Eclipse TE 2000-S, 40x). **(A)** PBMC and PMN rolling flux. **(B)** PBMC and PMN adhesion. Results are mean ± S.E.M. *p≤0.05, , **p≤0,01, ***p≤0,001 vs. corresponding value in the TDF group (Unpaired t test with Welch's correction).

PLATELET ADHESION TO THE ENDOTHELIUM WAS HIGHER IN ABC-GROUP THAN IN TDF-GROUP

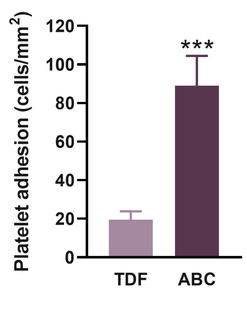

Figure 4. Platelet-endothelium interactions in HIV-infected patients. Suspensions of platelets from TDF-treated and ABC-treated HIV patients were drawn across a non-HIV human endothelial monolayer. To visualize platelets, they were labelled with anti-CD41-Alexa®488 antibody. Results are mean ± S.E.M. ***p≤0.001 vs. corresponding value in the TDF group (Unpaired t test with Welch's correction).

CONCLUSIONS

Treatment with ABC enhances leukocyte-endothelium interactions, thus promoting the **initial phases of the inflammatory process**. The drug also induces the adhesion of platelets to endothelial cells, an important step in **thrombus formation**. Our results give credence to the **increased rates of myocardial infarction observed in ABC-treated patients.**