Short communication

Differential effects of tenofovir/emtricitabine and abacavir/lamivudine on human leukocyte recruitment

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Background: The association of abacavir (ABC) with cardiovascular disease has led to HIV treatment guidelines favouring the combination of tenofovir/emtricitabine (TDF/FTC) over that of ABC/lamivudine (ABC/3TC). We have analysed the effects of plasma-relevant concentrations of TDF, FTC, ABC and 3TC, individually and in clinically employed combinations, on human leukocyte accumulation. The effects of ABC, 3TC, TDF and FTC on the expression of adhesion molecules were also evaluated.

Methods: Interactions between human leukocytes – specifically peripheral blood polymorphonuclear or mononuclear cells – and human umbilical vein endothelial cells were evaluated in a flow chamber reproducing in vivo conditions. The expression of adhesion molecules was analysed by flow cytometry.

Results: Concentrations of TDF, FTC or 3TC mimicking those in the plasma of patients did not have any effect on human leukocyte–endothelial cell interactions, while contrasting results were obtained with ABC. This distinct pattern was reproduced when the drugs were administered in combination; namely, ABC/3TC had a significant influence on rolling and adhesion while TDF/FTC did not. However, the effects produced by ABC alone did not differ when it was combined with 3TC, which suggests the former drug was responsible for the effects observed. ABC, 3TC, TDF and FTC did not modify the expression of endothelial adhesion molecules. Conversely, only ABC enhanced the expression of leukocyte CD11b/CD18 in neutrophils and monocytes.

Conclusions: Our results provide evidence that the combination TDF/FTC has a better vascular profile than ABC/3TC.

Introduction

Abacavir/lamivudine (ABC/3TC) and tenofovir/emtricitabine (TDF/FTC) are the most widely used nucleoside reverse transcriptase inhibitor (NRTI) associations in combined antiretroviral therapy [1]. Current clinical guidelines reflect a preference for the TDF/FTC combination due to the lower virological efficacy of ABC/3TC [1] and evidence, though not uniformly reproduced [2], that ABC (and not TDF) is associated with cardiovascular disease (CVD) [3,4]. This recommendation should however be viewed with caution, since a similar association between TDF/FTC and CVD may have gone undetected until now due to this combination having been commercialized for a shorter period of time.

The relationship between ABC and CVD cannot be explained by an effect on lipid and glucose metabolism. Indeed, the risk of CVD is reduced when therapy with this drug is discontinued, which points towards a direct and more acute mechanism such as vascular inflammation [3–5]. The role of vascular inflammation in CVD is widely acknowledged [5] and involves the accumulation in the vessel wall of both leukocytes and platelets as a consequence of interactions between adhesion molecules expressed on these cells and/or the endothelium [6,7]. In this circumstance, leukocytes roll along the wall of inflamed vessels before coming to a halt, after which they adhere and transmigrate. In a second phase, platelets bind to the endothelium and recruit other circulating platelets and leukocytes, thereby amplifying the thrombotic and atherosclerotic process.
We have recently demonstrated the capacity of ABC and didanosine (ddl), another purine analogue, to elicit leukocyte accumulation, an effect that was not observed with the pyrimidine analogues 3TC and zidovudine [8]. In the present study, we compare TDF/FTC and ABC/3TC, individually and in combination, on leukocyte accumulation. The effects of FTC, TDF, ABC and 3TC on the expression of endothelial and leukocyte adhesion molecules are also analysed.

Methods

We employed passage 1 human umbilical vein endothelial cells (HUVECs) harvested from umbilical cords [8]. Peripheral blood mononuclear (PBMC) or polymorphonuclear (PMN) cells were isolated from whole blood of healthy volunteers [8]. Samples were obtained from Hospital Clínico Universitario (Valencia, Spain) following approval from its ethical committee.

Adhesion assay under flow conditions

The parallel plate flow chamber model was used for these assays [9,10]. Coverslips (fibronectin [5 µg/ml]-coated) containing confluent HUVEC monolayers were inserted in the chamber (37°C) so that a portion (5×2.5 mm) was exposed to the flow. The chamber was mounted on an inverted microscope (Nikon Eclipse TE 2000-S, ×40; Amstelveen, the Netherlands) with a video camera (Sony Exware HAD; Koeln, Germany). PMNs or PBMCs were re-suspended in buffer (Dulbecco’s PBS with (DPBS+) or without (DPBS-) Ca²⁺ and Mg²⁺, endothelial cell growth media-2 culture media and fetal bovine serum (LONZA, Barcelona, Spain), human serum albumine (HSA, Albuminate 25%), RPMI1640 supplemented with 20 mM HEPES, HBSS, fibronectin and dextran (Sigma Chemical Co, Madrid, Spain); Ficoll-Paque TM Plus (GE Healthcare, Valencia, Spain), coverslips (Nunc, Thermo Fisher Scientific, Madrid, Spain), PBS, collagenase and trypsin (Gibco, Invitrogen, Barcelona, Spain), Immunoprep reagent (Beckman Coulter, Izasa, Barcelona, Spain), antiretrovirals (Sequoia Research Products, Pangbourne, UK) and fluorescein isothiocyanate- or phycoerythrin-conjugated antibodies (BD Bioscience, Madrid, Spain).

Statistics

One-way ANOVA with a Newman–Keuls post-test correction was employed for statistical analysis (mean ±SEM; n≥4; P<0.05).

Results

Leukocyte–endothelial cell interactions

FTC, TDF, 3TC or TDF/FTC did not modify rolling velocity, rolling flux or adhesion of PMN and PBMC (Figure 1). ABC and ABC/3TC induced a significant increase in rolling flux and adhesion while decreasing the rolling velocity of PMN and PBMC (Figure 1).

Expression of adhesion molecules

Leukocyte adhesion molecules were analysed in blood samples and endothelium molecules in confluent HUVECs [8]. Cells were treated with the NRTI (4 h, 37°C), incubated with saturating amounts of antibodies (20 min, 4°C, darkness), fixed and identified in a flow cytometer (EPICS XL-MCL cytometer; Coulter Electronics, Hialeah, FL, USA). Mean fluorescence intensity was employed as marker of expression of the respective epitope.

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Discussion

This is the first study to analyse the effects of the two most recommended NRTIs, TDF and FTC, on leukocyte accumulation. We evaluated the effects of both drugs individually and together, as they are administered in clinical practice, and compared them with those of the recommended alternative combination (ABC/3TC). Concentrations of TDF (0.5–1 μmol/l), FTC (5–10 μmol/l), ABC (10 μmol/l) or 3TC (10 μmol/l) or vehicle. Results are mean ± SD, n=4. *P<0.001 versus corresponding value in vehicle-treated group (ANOVA followed by Newman–Keuls test).

Comparison of the effects of emtricitabine (FTC), tenofovir (TDF), abacavir (ABC), lamivudine (3TC), TDF/FTC or ABC/3TC on (A) rolling velocity, (B) rolling flux and (C) adhesion of peripheral blood polymorphonuclear cells (PMN) and on (D) rolling velocity, (E) rolling flux and (F) adhesion of peripheral blood mononuclear cells (PBMC).

Human umbilical vein endothelial cells and leukocytes (PMNs or PBMCs) were treated (4 h) with FTC (5–10 μmol/l), TDF (0.5–1 μmol/l), ABC (10 μmol/l), 3TC (10 μmol/l), TDF/FTC (1/10 μmol/l), ABC/3TC (10/10 μmol/l) or vehicle. Results are mean ± SD, n=4. *P<0.001 versus corresponding value in vehicle-treated group (ANOVA followed by Newman–Keuls test).
The actions of ABC could be related to its chemical structure. Since leukocyte accumulation is induced only by cyclic purine analogues (ABC or ddI), and not by pyrimidine analogues (3TC, zidovudine, FTC) or the acyclic nucleotide TDF (current observations and [8]), we propose that ABC and ddI competitively inhibit the purine signalling cascade and increase levels of proinflammatory ATP. This would lead to augmentation of CD11b/CD18 and, consequently, of leukocyte–endothelial interactions. This mechanism is related to the one that seems to underlie activation of platelets by ABC, which involves inhibition of guanyllyl cyclase, followed by a decrease in cGMP and increased expression of P-selectin [17].

Clinical studies suggested a relationship between ABC (but not TDF/FTC) and CVD [3,4] and have implicated the expression of metabolic, inflammatory, procoagulant or endothelial markers in said relationship [18,19]. For instance, patients switching from ABC/3TC to TDF/FTC show improvements in both arterial stiffness and CVD markers, including c-reactive protein, interleukin (IL)-6, D-dimer and cholesterol [20]. Indeed, two studies have concluded that TDF has anti-inflammatory potential. One reported a significant improvement in the endothelial dysfunction associated with HIV-infection and decreased levels of sVCAM-1 and monocyte chemoattractant protein-1 in patients receiving TDF, but not in those on ABC [21]. The second described the capacity of TDF to undermine the release of cardiovascular-related inflammatory cytokines (IL-8 and MIP-1α) from human primary cells and to produce a shift in the IL-10/IL-12 balance towards an anti-inflammatory profile [22].

Given that CVD is the most prevalent cause of death among the normal aged population, any hint of an association with the drugs used in combined antiretroviral therapy is of great relevance. Our results should be interpreted with caution, but they do confirm recent clinical evidence that the combination TDF/FTC has a better vascular profile than that of ABC/3TC and endorse recent guidelines favouring the use of TDF/FTC.

## Acknowledgements

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### Table 1. Expression of endothelial adhesion molecules

<table>
<thead>
<tr>
<th>E-selectin</th>
<th>ICAM-1</th>
<th>VCAM-1</th>
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<tbody>
<tr>
<td>FTC</td>
<td>103.9 ±3.0</td>
<td>102.2 ±2.3</td>
</tr>
<tr>
<td>TDF</td>
<td>102.9 ±5.7</td>
<td>105.7 ±7.4</td>
</tr>
<tr>
<td>ABC</td>
<td>109.1 ±5.6</td>
<td>109.0 ±5.6</td>
</tr>
<tr>
<td>3TC</td>
<td>109.0 ±5.6</td>
<td>109.0 ±5.6</td>
</tr>
</tbody>
</table>

Data is adhesion molecules in human umbilical vein endothelial cells (HUVECs), percentage versus control. HUVECs were treated with emtricitabine (FTC; 10 μmol/l), tenofovir (TDF; 1 μmol/l), abacavir (ABC; 10 μmol/l), lamivudine (3TC; 10 μmol/l) or vehicle (4 h) and were analysed by flow cytometry as described in Methods. Fluorescein isothiocyanate- or phycoerythrin-fluorescence values are expressed as a percentage of the mean fluorescence intensities of control cells (100%). Results are mean±SEM of n=4–6 experiments. ANOVA followed by Newman–Keuls test was performed. ICAM-1, intercellular adhesion molecule–1; VCAM-1, vascular cell adhesion molecule–1.
Table 2. Expression of leukocyte adhesion molecules CD11a, CD11b, CD18 and CD49d integrin subunits on neutrophils and monocytes

<table>
<thead>
<tr>
<th>Adhesion molecules in leukocytes</th>
<th>FTC</th>
<th>TDF</th>
<th>ABC</th>
<th>3TC</th>
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</thead>
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<tr>
<td>Neutrophils, % versus control</td>
<td></td>
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<tr>
<td>CD11a</td>
<td>96.8 ±2.4</td>
<td>96.2 ±2.6</td>
<td>102.0 ±2.6</td>
<td>97.9 ±3.1</td>
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<tr>
<td>CD11b</td>
<td>96.3 ±0.3</td>
<td>103.3 ±1.3±</td>
<td>106.0 ±1.8±</td>
<td>96.3 ±0.1</td>
</tr>
<tr>
<td>CD18</td>
<td>96.8 ±4.1</td>
<td>96.3 ±5.7</td>
<td>122.4 ±7.0±</td>
<td>92.18 ±9.2</td>
</tr>
<tr>
<td>CD49d</td>
<td>99.7 ±0.3</td>
<td>99.8 ±0.3</td>
<td>99.5 ±0.5</td>
<td>94.9 ±5.5</td>
</tr>
<tr>
<td>Monocytes, % versus control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD11a</td>
<td>99.1 ±4.2</td>
<td>105.6 ±4.6</td>
<td>106.9 ±6.6</td>
<td>102.8 ±8.0</td>
</tr>
<tr>
<td>CD11b</td>
<td>70.9 ±10.3</td>
<td>85.0 ±18.9</td>
<td>143.9 ±9.2±</td>
<td>87.5 ±17.7</td>
</tr>
<tr>
<td>CD18</td>
<td>99.2 ±0.6</td>
<td>92.5 ±2.5</td>
<td>119.3 ±6.4±</td>
<td>92.0 ±5.3</td>
</tr>
<tr>
<td>CD49d</td>
<td>102.4 ±3.2</td>
<td>97.8 ±3.5</td>
<td>100.1 ±5.1</td>
<td>102.6 ±0.4</td>
</tr>
</tbody>
</table>

Whole blood was treated with emtricitabine (FTC; 10 µmol/l), tenofovir (TDF; 1 µmol/l), abacavir (ABC; 10 µmol/l), lamivudine (3TC;10 µmol/l) or vehicle (4 h) and was analysed by flow cytometry, as described in Methods. Fluorescein isothiocyanate- or phycoerythrin-fluorescence values are expressed as a percentage of the mean fluorescence intensities of control cells (100%). Results are mean ±SEM of n=4–6 experiments. *P<0.01 versus corresponding value in vehicle-treated group (ANOVA followed by Newman–Keuls test).

CDP performed the research, and was assisted by SO, SC and MM-C. CDP and AA analysed the data. JVE and AA designed the research and wrote the manuscript.

Disclosure statement
The authors declare no competing interests.

References