Efavirenz induces interactions between leucocytes and endothelium through the activation of Mac-1 and gp150,95

Samuel Orden1, Carmen De Pablo1, Cesar Rios-Navarro1, Maria Angeles Martinez-Cuesta1, Jose E. Peris2, Maria D. Barrachina1, Juan V. Esplugues1,3 and Angeles Alvarez1,4*

1Departamento de Farmacología y CIBERehd, Facultad de Medicina, Universidad de Valencia, Valencia, Spain; 2Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain; 3FISABIO-Fundación Hospital Universitario Dr. Peset, Valencia, Spain; 4Fundación General Universidad de Valencia, Valencia, Spain

*Corresponding author. Departamento de Farmacología, Facultad de Medicina, Universidad de Valencia, Avda. Blasco Ibáñez 15–17, 46010 Valencia, Spain. Tel: +34-96-3864898; Fax: +34-96-3983879; E-mail: angeles.alvarez@uv.es

Received 23 May 2013; returned 19 July 2013; revised 1 October 2013; accepted 1 November 2013

Objectives: The potential cardiovascular (CV) toxicity associated with combined antiretroviral therapy (cART) has been attributed mainly to the nucleoside reverse transcriptase inhibitors abacavir and didanosine. However, the other two components of cART—non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs)—may also be implicated, either directly or by influencing the action of the other drugs. This study evaluates the acute direct effects of the NNRTIs efavirenz and nevirapine and one of the most widely employed PIs, lopinavir, on leucocyte–endothelium interactions, a hallmark of CV disease.

Methods: Drugs were analysed in vitro in human cells (interactions of peripheral blood polymorphonuclear or mononuclear cells with human umbilical vein endothelial cells) using a flow chamber system, and in vivo in rat mesenteric vessels by means of intravital microscopy. The expression of adhesion molecules in leucocytes and endothelial cells was studied by flow cytometry, and the role of these molecules in white cell recruitment was evaluated by pre-treating human cells or rats with blocking antibodies.

Results: Efavirenz and nevirapine, but not lopinavir, increased the rolling flux and adhesion of leucocytes in vitro and in vivo while inducing emigration in rat venules. Efavirenz, but not nevirapine, augmented the levels of CD11b, CD11c and CD18 in neutrophils and monocytes. The actions of efavirenz, but not of nevirapine, were reversed by antibodies against Mac-1 (CD11b/CD18), gp150,95 (CD11c/CD18) or ICAM-1 (CD54).

Conclusions: NNRTIs, but not PIs, interfere with leucocyte–endothelium interactions. However, differences between efavirenz and nevirapine suggest a specific CV profile for each compound.

Keywords: nevirapine, lopinavir, leucocyte–endothelium interactions, cardiovascular

Introduction

There is growing awareness of the chronic toxic effects induced by the lifelong administration of combined antiretroviral therapy (cART) to HIV-infected patients, particularly in relation to the cardiovascular (CV) diseases that accompany ageing.1 cART involves the administration of at least three drugs: typically, two nucleoside reverse transcriptase inhibitors (NRTIs) plus either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI).2,3 Although CV toxicities have been attributed largely to the NRTI component of cART, in particular abacavir and didanosine,4–6 ar o l eh a s also been proposed for NNRTIs and, especially, PIs.1,7,8

Efavirenz is the most widely prescribed NNRTI; the majority of guidelines recommend its use in conjunction with tenofovir and emtricitabine or with abacavir and lamivudine as the initial treatment for HIV.2,3 Although generally considered a safe drug, there is evidence that efavirenz could also be implicated in the onset of some CV disorders. The exposure of patients to efavirenz is associated with endothelial dysfunction9 and with increases in low-density lipoprotein,10 F2 isoprostane and high-sensitivity C-reactive protein,1 which are markers of metabolic parameters, oxidative stress and vascular inflammation, respectively, and risk factors for CV conditions in all three cases. Furthermore, in isolated human cells, efavirenz has been shown to induce acute direct mitochondrial toxicity11 and to increase superoxide anion and vascular permeability by reducing levels of the proteins that maintain endothelium–endothelium junctional structures.12,13

An increase in vascular permeability coupled with leucocyte infiltration is a hallmark of the inflammation that underlies vascular diseases and the HIV infection itself.14 This recruitment of white
cells involves a sequential cascade of adhesive interactions between leucocytes and endothelial cells that begins with enhanced rolling and leads to the adhesion and subsequent endothelial transmigration of these white cells in a process mediated by the adhesion molecules present on both cell populations. The aim of the present study was to evaluate the direct effect of efavirenz in the early phases of responses after exposure: leucocyte–endothelial cell interactions both in vitro and in vivo. We extended our analysis to include nevirapine, the other widely employed NNRTI, and lopinavir, a PI commonly used as an alternative to efavirenz in HIV therapy.

Materials and methods

Cell culture of human umbilical vein endothelial cells (HUVECs) and leucocyte isolation

HUVECs were harvested from freshly obtained umbilical cords as previously described, and passage 1 cells were subsequently employed in the experiments. Human peripheral blood polymorphonuclear (PMN) cells or peripheral blood mononuclear cells (PBMCs) were isolated from whole blood drawn from healthy volunteers and anticoagulated with sodium citrate. Leucocytes and HUVECs were treated independently (4 h, 37°C) with efavirenz (10–25 μM), nevirapine (10–50 μM), lopinavir (10–25 μM), control (without vehicle) or vehicle [efavirenz and lopinavir were dissolved in methanol and nevirapine in azide water (pH ≤ 3)]. The aforementioned concentrations were employed because they mimic clinically relevant plasma levels. The Medical Ethics Committee of the Hospital Clínico Universitario de Valencia approved the study, and the experiments were conducted in accordance with the Declaration of Helsinki after obtaining the written informed consent of each participant.

Dynamic adhesion assay under flow conditions

The characteristics of the parallel-plate flow chamber model used in this study have been described previously. In brief, coverslips [coated with fibronectin (5 μg/mL)] containing confluent HUVEC monolayers were inserted in the chamber (37°C) and a portion (5×25 mm) was exposed to the flow. The chamber was mounted on an inverted microscope (Nikon Eclipse TE 2000-S, x40, Amstelveen, The Netherlands) with a video camera (Sony Exware HAD, Koeln, Germany). PMN cells or PBMCs were resuspended in Dulbecco’s PBS with Ca²⁺ and Mg²⁺ buffer containing 20 mM HEPES and 0.1% human serum albumin at 1×10⁶ or 0.5×10⁶ cells/mL, respectively, and were drawn across the monolayer (flow rate 0.36 mL/min, shear stress 0.7 dyne/cm²). Images of a single field were recorded for 5 min and leucocyte parameters were determined. Rolling flux was calculated by counting the number of cells rolling across 100 μm² of the monolayer during a 1 min period. The velocities of 20 consecutive leucocytes in the field of focus were determined by measuring the time required to travel 100 μm. Adhesion was determined after 5 min of perfusion by the analysis of 5–10 high-power (>40×) fields. Leucocytes were considered to be adherent after 30 s of stable contact with the monolayer.

Flow cytometry

The expression of human leucocyte adhesion molecules was analysed in blood samples and that of endothelium molecules was quantified in confluent HUVECs. Blood samples and endothelial cells were treated (4 h, 37°C) with the different antiretroviral drugs and then incubated with saturating amounts of the antibodies (20 min, 4°C, in darkness). Subsequently, the samples were fixed and identified in a flow cytometer (FACScalibur Flow Cytometer, BD, Madrid, Spain). Surface antigen expression [FITC or phycoerythrin (PE) fluorescence] was analysed in granulocytes, monocytes and lymphocytes, which were identified by their specific features of size [forward-angle light scatter (FSI) and granularity [side-angle light scatter (SSI)]. HUVECs were also recognized by their FS and SS characteristics. Median fluorescence intensity (FITC or PE) was employed as a marker of the expression of the respective epitope.

Intravital microscopy

Leucocyte–endothelial cell interactions were evaluated in fasted male Sprague–Dawley rats (200–250 g) following a standard experimental technique. In brief, rats were anaesthetized with sodium pentobarbital (65 mg/kg, intraperitoneally [ip]) and, following a midline abdominal incision, a segment of the mid-jejunum was exteriorized and placed on an optically clear viewing pedestal at 37°C for tissue transillumination. The exposed mesentery was visualized using an orthoscopic microscope (Nikon Optiphot-2, SMZ1, Nikon, Badhoevedorp, The Netherlands) equipped with a ×20 objective lens (Nikon SLDW) and ×10 eyepieces, during which time it was continuously superfused with bicarbonate buffer saline (pH 7.4, 37°C, 2 mL/min). A video camera (Sony SSC-C350P; Sony, Koeln, Germany) mounted on the microscope projected images onto a colour monitor (Sony Trinitron PVM-14N2E), and the images were captured on videotape (Sony SVT-S3000P) for playback analysis (final magnification of the video screen: ×1300). Single unbranched mesenteric venules were selected and their diameters (25–40 μm) measured online using a video caliper (Microcirculation Research Institute, Texas A&M University, College Station, TX, USA). Centerline red blood cell velocity (Vrbc) was quantified online with an optical Doppler velocimeter (Microcirculation Research Institute). Blood flow and wall shear rate (γ) were calculated as previously described, and systemic arterial blood pressure was monitored. Numbers of rolling, adherent and emigrated leucocytes were determined offline during playback analysis of videotaped images. Rolling flux was assessed by counting the number of leucocytes passing a reference point in the vessel per minute. Leucocyte rolling velocity (Vroll) was calculated by measuring the time required for these cells to travel along 100 μm of the venule and was expressed as μm/s. A leucocyte was considered to have adhered to the endothelium if it remained stationary for ≥ 30 s, and the numbers of these leucocytes were expressed per 100 μm of vessel. Leucocyte emigration was evaluated as the total number of interstitial leucocytes per field. In some cases, the area of the mesentery selected for the experiment was excised, fixed with paraformaldehyde (4% in PBS, pH 7.4) and stained with haematoxylin and eosin. The preparation was subsequently observed under a clear field microscope (×63), and the infiltrated leucocytes were counted (number per 2.5×10⁻⁵ cm²) and classified morphologically as PMN leucocytes, macrophages and lymphocytes by an observer who was unaware of the treatment in question. Animals were injected (2.5 mL, ip) with saline, vehicles (methanol or azide water) or one of the following antiretroviral drug solutions: efavirenz (10–25 μM, equivalent to 40–100 μg/kg), nevirapine (10–50 μM; 35–165 μg/kg) or lopinavir (10–25 μM; 80–200 μg/kg). Images (5 min period) were recorded 4 h later in order to allow enough time for the process of leucocyte emigration to initiate and were then evaluated by an observer who was blind to the treatment. The ip injection of drug solutions is a usual practice in intravitral microscopy, as it allows longer incubation periods than superfusion and is, thus, more suited to lengthy time-course studies such as the present one. All doses were representative of plasma concentrations in patients. Care was taken at each stage of the experiment to avoid any suffering of the animals. Procedures followed Spanish law concerning the use of experimental animals and were approved by the Ethics Committee of the Faculty of Medicine of the University of Valencia (Spain).

Functional role of adhesion molecules

This was assessed in both in vitro and in vivo settings by blocking the adhesion molecules with specific antibodies. For the in vitro experiments,
and prior to treatment with efavirenz, PMN cells and PBMCs were pre-treated with anti-lymphocyte function-associated antigen-1 (CD11a-LFA-1; 10 μg/mL), anti-macrophage-1 antigen (CD11b-Mac-1; 20 μg/mL), anti-alpha-X integrin (CD11c-gp150,95; 10 μg/mL), anti-β2 integrins (CD18; 10 μg/mL) or control antibodies (IgG1; 10 μg/mL) for 20 min at 4°C in darkness. Prior to drug administration, HUVEC monolayers were pre-treated with anti-intercellular adhesion molecule-1 (ICAM-1-CD54; 20 μg/mL) or control antibodies (IgG1; 10 μg/mL) for 30 min at 37°C. The antibodies were assayed at previously described doses.25 27–29 In the in vivo experiments, antibodies were injected through the tail vein 30 min before efavirenz or nevirapine administration, as previously described,26,27–29 at doses that have been shown to block the in vivo function of the adhesion molecules analysed in the current study (2 mg/kg for WT-5 (anti-rat Mac-1, CD11b), and 1 mg/kg for WT-3 (anti-β2 integrins, CD18) and for 1A29 (anti-rat ICAM-1, CD54)) or with control antibodies (2 mg/kg for IgA and 1 mg/kg for IgG1).25,26 To rule out a direct effect of the monoclonal antibodies (MAbs) on circulating leucocytes, portal blood samples were obtained and their number evaluated after the intravital measurements.

Materials

Blocking antibodies, FITC-conjugated antibodies and lysis solution (BD Bioscience, Madrid, Spain); Dulbecco’s PBS with or without Ca²⁺ and Mg²⁺, EGM-2 culture media and fetal bovine serum (Lonza, Barcelona, Spain); human serum albumin (albuminate 25%), RPMI 1640 supplemented with human serum albumin (Sigma Chemical Co., Madrid, Spain); eosin (Panreac, Barcelona, Spain); Ficoll-Paque™ Plus (GE Healthcare, Valencia, Spain); coverslips (Nunc, Thermo Fisher Scientific, Madrid, Spain); PBS, collagenase and trypsin (Gibco, Invitrogen, Barcelona, Spain); antiretroviral drugs (Sequoia Research Products, Pangbourne, UK); Sprague–Dawley rats (Charles River Laboratories, Barcelona, Spain); and pentobartol (Guinama, Valencia, Spain).

Results

Leucocyte–endothelium cell interactions

In human cells in vitro, efavirenz and nevirapine induced a decrease in rolling velocity (Figure 1a and b) and an increase in the rolling flux (Figure 1c and d) and adhesion (Figure 1e and f) of both PMN cells and PBMCs. None of these interactions was generated by any of the assayed doses of efavirenz, nevirapine or lopinavir had any effect on the adhesion molecules of lymphocytes (data not shown) or human endothelial cells (Table S1, available as Supplementary data at JAC Online).

Role of adhesion molecules in NNRTI-induced leucocyte–endothelium interactions

Flow cytometry revealed that treatment with efavirenz significantly and dose-dependently augmented the expression of CD11b, CD11c and CD18 on both neutrophils and monocytes in human blood, while that of CD11a, CD49d and CD62L (L-selectin) was not affected (Table S1, available as Supplementary data at JAC Online). Neither nevirapine nor lopinavir modified the expression of the adhesion molecules analysed on neutrophils or monocytes (Table S1, available as Supplementary data at JAC Online). None of the assayed doses of efavirenz, nevirapine or lopinavir had any effect on the adhesion molecules of lymphocytes (data not shown) or human endothelial cells (Table S1, available as Supplementary data at JAC Online).

The functional implication of adhesion molecules was assessed with specific antibodies. In vitro, the effects of efavirenz on interactions between human leucocytes (PMN cells or PBMCs) and the endothelium were completely reversed by blocking Mac-1 (CD11b), gp150,95 (CD11c), β2 integrins (CD18) or their ligand ICAM-1 (CD54), but not by neutralizing LFA-1 (CD11a) (Figure 4). These in vitro results were confirmed in rat post-capillary venules in vivo; namely, the effects of efavirenz were not apparent when animals were pre-treated with antibodies against Mac-1 (CD11b), β2 integrins (CD18) or ICAM-1 (CD54) (Figure 5). However, the responses induced by nevirapine were not modified when animals were pre-treated with any of the abovementioned antibodies. Neither haemodynamic parameters nor systemic leucocyte count was affected by any of the antibodies employed (data not shown).

Discussion

This study shows that efavirenz, the most commonly employed NNRTI in HIV therapy, induces leucocyte recruitment through the interaction of certain β2 integrins with ICAM-1. We obtained our results in two experimental models: (i) human cells in vitro; and (ii) rat mesenteric venules in vivo. The former is a dynamic experimental setting in which human leucocytes flow over a monolayer of HUVECs in a way that reproduces the rolling and adhesion processes that precede the formation of an inflammatory focus.35,40 The latter permits a more detailed analysis of the inflammatory outcome by evaluating the emigration of leucocytes from living vessels.31,41 A combination of the two experimental approaches allows for a more comprehensive analysis of the potentially proinflammatory vascular effects of drugs.

Concentrations of efavirenz (10–25 μM) or nevirapine (10–50 μM) mimicking those found in patients (3–13 and 10–25 μM, respectively)27,29,30 induced human leucocyte (PMN or PBMC)–endothelial cell interactions in vitro. Although the functional effect of efavirenz on PBMC–endothelial cell interactions seemed to be weak, the expression of CD11b and CD11c molecules was particularly evident on monocytes, while almost absent in lymphocytes.42 In our opinion this discrepancy results from the fact that, of the total population of PBMCs superfused through the flow chamber, only a small proportion were monocytes (~10%); thus, their reactivity would be obscured by the 90% of lymphocytes that would not adhere to the endothelium due to their lack (both constitutively or after efavirenz incubation) of CD11b or CD11c.42 Lopinavir (10–25 μM in our experiments; 7–17 μM in patients),21 a PI, had no

Statistical analysis

One-way analysis of variance (ANOVA) with a Newman–Keuls post-test correction was employed for statistical analysis (mean ± SEM, n ≥ 4, P < 0.05).
Figure 1. Effects of efavirenz, nevirapine and lopinavir on PMN–endothelial cell interactions and PBMC–endothelial cell interactions. HUVECs and leucocytes (PMN cells or PBMCs) were incubated (4 h) with efavirenz (10–25 μM), nevirapine (10–50 μM), lopinavir (10–25 μM) or the corresponding vehicles (efavirenz and lopinavir in methanol; nevirapine in azide water pH ≤ 3). After assembling the flow chamber, PMN rolling velocity (a), rolling flux (c) and adhesion (e) and PBMC rolling velocity (b), rolling flux (d) and adhesion (f) were quantified. Results are means ± SEM, n = 4–6. *P < 0.05 versus control (C) group, **P < 0.01 versus control group and ^^P < 0.01 versus methanol- or azide water-treated group (ANOVA followed by Newman–Keuls test). EFV, efavirenz; NVP, nevirapine; LPV, lopinavir.
Figure 2. Effects of efavirenz, nevirapine and lopinavir on leucocyte responses in rat mesenteric post-capillary venules. Animals were treated (ip) with efavirenz (10–25 µM), nevirapine (10–50 µM), lopinavir (10–25 µM), control (saline) or the corresponding vehicles (efavirenz and lopinavir in methanol; nevirapine in azide water pH ≤ 3). Four hours later, responses of leucocyte rolling velocity (a), rolling flux (b), adhesion (c) and emigration (d) were quantified. Results are means ± SEM, n = 4–5. *P < 0.05 or **P < 0.01 versus corresponding value in saline-treated group and ^P < 0.05 or ^^P < 0.01 versus corresponding value in methanol- or azide water-treated group (ANOVA followed by Newman–Keuls test). EFV, efavirenz; NVP, nevirapine; LPV, lopinavir; C, control.
effect on any of the parameters evaluated. As a whole, these findings challenge previous perceptions of NNRTIs as relatively CV-friendly drugs and of PIs as potentially CV-toxic molecules.7,43

The results obtained in vitro were reproduced and expanded in the in vivo model; in addition to inducing leucocyte rolling and adhesion, both efavirenz and nevirapine (but not lopinavir) caused a significant emigration of white cells within post-capillary venules. This is of great relevance, as the movement of leucocytes towards inflamed tissue is considered the point of no return in the vascular inflammatory cascade, while the preceding phases (rolling and adhesion) are reversible.64 The level of emigration induced by efavirenz was significantly higher than that produced by nevirapine (quantified by intravital microscopy and haematoxylin/eosin staining), which is compatible with the fact that nevirapine (and indeed lopinavir) had no effect on leucocytes or endothelial adhesion molecules. By contrast, efavirenz produced a selective up-regulation of the β2 integrins Mac-1 (CD11b/CD18) and gp150,95 (CD11c/CD18) on neutrophils and monocytes, but not of the endothelial molecules E-selectin, ICAM-1 or VCAM-1.

When the functional implication of adhesion molecules was evaluated, the interactions induced by efavirenz were reversed by pre-treatment with antibodies against CD11b, CD18 or ICAM-1 (CD54), both in vitro and in vivo. The role of CD11c was evaluated in human cells only because of the unavailability of a commercially marketed rat antibody against this molecule. Our analysis of the role of adhesion molecules in the effects of efavirenz on the interplay between leucocytes and the endothelium strongly suggests that both β2 integrins—gp150,95 (CD11c/CD18) and Mac-1 (CD11b/CD18)—interact with their endothelial ligand ICAM-1 (CD54). Although the involvement of CD11b and CD11c in adhesion and emigration would be expected, rolling was initially considered to be mediated by selectins and/or VLA-4/VCAM-1.62 However, there is growing evidence that β2 integrins also mediate leucocyte rolling in close dependence on their conformational state.45,46

β2 Integrins (CD18) are cell adhesion receptors that share the same CD18 β chain but exhibit a distinctive α chain. The family includes four known types: αL (CD11a, LFA-1), αM (CD11b, Mac-1), αX (CD11c, gp95,150) and αD (CD11d).42 The expression of CD11b and CD11c in lymphocytes is limited, but they are mobilized from intracellular secretory vesicles to the cell surface of neutrophils and monocytes within minutes of stimulation.64 Their main ligand is ICAM-1, which is constitutively expressed on the surface of the vascular endothelium.62 CD11c has already been implicated in phagocytosis, antigen presentation by dendritic cells and the inflammatory response.66 In addition to its role in mediating the adherence of neutrophils and monocytes to the vessel wall, Mac-1 (CD11b) has been proposed as a key link between cellular adhesion and thrombosis67 by mediating the engagement of platelets.68

The actions of nevirapine were not mediated by CD11b, CD11c or CD18, yet it did increase leucocyte rolling flux to a level similar to that

Figure 3. Leucocyte infiltration in the mesentery of rats treated with efavirenz or nevirapine. Animals were treated (ip) with efavirenz (15 μM), nevirapine (25 μM), control (saline) or the corresponding vehicles (efavirenz in methanol; nevirapine in azide water pH ≤ 3) during the 4 h period. The mesentery selected for intravital experiments was excised, fixed with paraformaldehyde and stained with haematoxylin and eosin, and the number of infiltrated leucocytes (neutrophils, monocytes or lymphocytes) was counted in an area of 2.5 x 10^-4 cm² in animals treated with methanol (a), 15 μM efavirenz (b), azide water (c) and 25 μM nevirapine (d). Arrows denote examples of infiltrated neutrophils. Bar = 50 μm. EFV, efavirenz; NVP, nevirapine.
Figure 4. Role of Mac-1, gp150,95 and ICAM-1 in PMN–endothelial cell interactions and PBMC–endothelial cell interactions induced by efavirenz. HUVECs and leucocytes (PMN cells or PBMCs) were treated (4 h) with efavirenz (15 μM) or its vehicle (methanol). Some PMN cells or PBMCs were pre-treated with anti-CD11a MAb, anti-CD11b MAb, anti-CD11c MAb or anti-CD18 MAb, and some HUVECs were pre-treated with anti-ICAM-1 MAb 30 min before treatment with efavirenz. After assembling the flow chamber, PMN rolling velocity (a), rolling flux (c) and adhesion (e) and PBMC rolling velocity (b), rolling flux (d) and adhesion (f) were quantified. Results are means ± SEM, n=4–5. **P<0.01 versus corresponding value in control (C) group, ^^P<0.01 versus corresponding value in methanol-treated group and +++P<0.01 versus corresponding value in efavirenz-treated group (ANOVA followed by Newman–Keuls test). EFV, efavirenz.
Figure 5. Role of Mac-1 and ICAM-1 in efavirenz- or nevirapine-induced leucocyte–endothelium interactions in rat mesenteric post-capillary venules. Rats were treated (ip) with efavirenz (15 μM), nevirapine (25 μM), control (saline) or the corresponding vehicles (efavirenz in methanol; nevirapine in azide water pH ≤ 3). Some animals were pre-treated (intravenously) with anti-CD11b MAb, anti-CD18 MAb, anti-ICAM-1 or the corresponding control MAbs 30 min before administration of efavirenz or nevirapine. Four hours later, responses of leucocyte rolling velocity (a), rolling flux (b), adhesion (c) and emigration (d) were quantified. Results are means ± SEM, n=4. **P<0.01 versus corresponding value in saline-treated group, ^^P<0.01 versus corresponding value in methanol- or azide water-treated group and +++P<0.01 versus corresponding value in efavirenz-treated group (ANOVA followed by Newman–Keuls test). EFV, efavirenz; NVP, nevirapine; C, control.
induced by efavirenz while influencing less adhesion and particularly emigration. One possible explanation for this differential profile could be that nevirapine activates other families of adhesion molecules, such as P-selectin, which are implicated mainly in the rolling process.\textsuperscript{52}

Although the clinical translation of our results should be contemplated with caution, we believe they provide strong evidence that NNRTIs, and in particular efavirenz, alter leucocyte–endothelial interactions. As a whole, our findings point to an effect by which efavirenz activates leucocytes but not endothelial cells. This contrasts with reports suggesting that efavirenz-containing regimens are responsible for impaired endothelial function in patients,\textsuperscript{9} or that, in vitro, this drug induces an increase in the levels of superoxide anions responsible for the endothelial damage and altered endothelial junctions that restrict vascular permeability.\textsuperscript{12} Nevertheless, it should be pointed out that the period of exposure to efavirenz was substantially shorter in the present study than in the reports in question. Furthermore, since HIV infection is itself characterized by an increase in endothelial adhesion molecules, the profile of which (ICAM-1, VCAM-1 and E-selectin)\textsuperscript{49} differs from that induced by efavirenz, it is feasible that the effects of efavirenz and those of the virus are cumulative in patients. In other words, the virus may cause endothelial activation whereas efavirenz stimulates white cells. Given the increasing longevity of HIV-infected patients, and taking into account that CV illnesses are the most frequent cause of death among the non-HIV-infected ageing population, a potential relationship between the compounds used in CART and CV toxicity is of considerable importance.

**Funding**

This work was supported by Ministerio de Ciencia e Innovación (grant numbers SAF2010-16030 and SAF2010-20231), Ministerio de Sanidad y Consumo (grant number PI11/00327), CIBERehd (grant number CB06/04/0071) and Generalitat Valenciana (grant numbers PROMETEO/2010/060, ACOMP/2013/147 and ACOMP/2013/236). S. O. was funded by Universidad de Valencia (VLC–CAMPUS grant) and C. d. P. by Ministerio de Ciencia e Innovación (FPI grant number BES-2008-004338) and by Fundación Juan March (fellowship number BMR/09/003 2009; and 13 program).

**Transparency declarations**

None to declare.

**Author contributions**

S. O. performed the research, C. d. P., C. R.-N., M. A. M.-C., J. E. P. and M. D. B. helped perform the research, S. O. and A. A. analysed the data, A. A. conceived the study and J. V. E. and A. A. designed the research and wrote the paper.

**Supplementary data**

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

**References**


