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

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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 a role has also been proposed for NNRTIs and, especially, PIs.5,7,8 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,11 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 toxicity12 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 adhesion and subsequent endo-
thelial transmigration of these white cells in a process mediated by
the adhesion molecules present on both cell populations.\textsuperscript{15,16} The
aim of the present study was to evaluate the direct effect of efavir-
enz in the early phases of responses after exposure: leucocyte–
endothelial cell interactions both \textit{in vitro} and \textit{in vivo}. We extended
our analysis to include nevirapine, the other widely employed
NNRTI, and lopinavir, a PI commonly used as an alternative to efa-
virenz in HIV therapy.

\section*{Materials and methods}

\subsection*{Cell culture of human umbilical vein endothelial cells (HUVECs) and leucocyte isolation}

HUVECs were harvested from freshly obtained umbilical cords as previously
described,\textsuperscript{17,18} 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.\textsuperscript{5} Leucocytes and HUVECs were treated independently (4 h, 37
°C) and a portion (5\% x 25 mm) was exposed to
methanol and nevirapine in azide water (pH ≤ 3). The aforementioned
concentrations were employed because they mimic clinically relevant
plasma levels.\textsuperscript{12,19–21} 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.

\subsection*{Dynamic adhesion assay under flow conditions}

The characteristics of the parallel-plate flow chamber model used in this
study have been described previously.\textsuperscript{2,6,22} In brief, coverslips [coated with
fibronectin (5 \(\mu\)g/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 Ca\textsuperscript{2+} and Mg\textsuperscript{2+} buffer containing 20 \textit{mM} HEPES and
0.1\% human serum albumin at 1 × 10\textsuperscript{6} or 0.5 × 10\textsuperscript{6} cells/mL, respectively,
and were drawn across the monolayer (flow rate 0.36 mL/min, shear
stress 0.7 dyne/cm\textsuperscript{2}). 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 \(\mu\)m\textsuperscript{2} 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 \(\mu\)m. Adhesion was determined after 5 min of perfusion by the analysis
of 5–10 high-power (>x40) fields. Leucocytes were considered to be adherent
after 30 s of stable contact with the monolayer.

\subsection*{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 Cyt-
ometer, BD, Madrid, Spain).\textsuperscript{23,24} Surface antigen expression [FITC or phyco-
erythrin (PE) fluorescence] was analysed in granulocytes, monocytes and
lymphocytes, which were identified by their specific features of size
(forward-angle light scatter (FS)) and granularity [side-angle light scatter
(SS)]. 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.

\section*{Intravital microscopy}

Leucocyte–endothelial cell interactions were evaluated in fasted male
Sprague–Dawley rats (200–250 g) following a standard experimental
\textsuperscript{technique.\textsuperscript{25,26}} In brief, rats were anaesthetized with sodium pentobarbital
(65 mg/kg, intraperitoneally [ip]) and, following a midline abdominal inci-
sion, a segment of the mid-jejunum was exteriorized and placed on an opti-
cally 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 x20 objective lens (Nikon SLDW) and x10 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: x1300). Single unbranched mesenteric venules were
selected and their diameters (25–40 \(\mu m\)) measured online using a video
caliper (Microcirculation Research Institute, Texas A&M University, College
Station, TX, USA). Centerline red blood cell velocity (\(V_{rbc}\)) was quantified
online with an optical Doppler velocimeter (Microcirculation Research Insti-
tute). Blood flow and wall shear rate (\(\gamma\)) were calculated as previously
described,\textsuperscript{27} and systemic arterial blood pressure was monitored.
Numbers of rolling, adherent and emigrated leucocytes were determined
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 (\(V_{rloe}\)) was calculated
by measuring the time required for these cells to travel along 100 \(\mu\)m of
the venule and was expressed as \(\mu\)m/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 \(\mu\)m 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 subse-
quently observed under a clear field microscope (x63), and the infiltrated
leucocytes were counted (number per 2.5 × 10\textsuperscript{4} cm\textsuperscript{2}) and classified mor-
phologically as PMN leucocytes, macrophages and lymphocytes by an ob-
server who was unaware of the treatment in question.\textsuperscript{16} 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 \(\mu\)M, equiva-
 lent to 40–100 \(\mu\)g/kg), nevirapine (10–50 \(\mu\)M; 35–165 \(\mu\)g/kg) or lopinavir
(10–25 \(\mu\)M; 80–200 \(\mu\)g/kg). Images (5 min period) were recorded 4 h later
in order to allow enough time for the process of leucocyte emigration to initi-
ate and were then evaluated by an observer who was blind to
the treatment. The ip injection of drug solutions is a usual practice in intravital
microscopy,\textsuperscript{23,28–30} as it allows longer incubation periods than super-
fusion\textsuperscript{11} and is, thus, more suited to lengthy time-course studies such as
the present one. All doses were representative of plasma concentrations
in patients.\textsuperscript{12,19–21} Care was taken at each stage of the experiment to
avoid any suffering of the animals. Procedures followed Spanish law con-
cerning the use of experimental animals and were approved by the Ethics
Committee of the Faculty of Medicine of the University of Valencia (Spain).

\section*{Functional role of adhesion molecules}

This was assessed in both \textit{in vitro} and \textit{in vivo} settings by blocking the
adhesion molecules with specific antibodies. For the \textit{in vitro} experiments,
and prior to treatment with efavirenz, PMN cells and PBMCs were pre-treated with anti-lymphocyte function-associated antigen-1 (CD11c–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-beta integrins (CD18; 10 μg/mL) or control antibodies (IgG1; 10 μg/mL) for 20 min at 4 °C in darkness. Prior to drug administration, HUVECs 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.32–35 For the in vivo experiments, antibodies were injected through the tail vein 30 min before efavirenz or nevirapine administration, as previously described,36,37 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-rat beta 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).23,38 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 (albuminurate 25%), RPMI 1640 supplemented with 20 mM HEPES, HBSS, fibronectin, dextran and haematoxylin (Sigma Chemical Co., Madrid, Spain); eosin (Panreac, Barcelona, Spain); Ficol-PaqueTM 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 pentobarbital (Guinama, Valencia, Spain).

**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).

**Results**

**Leucocyte–endothelial 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 abovementioned antibodies. Neither haemodynamic parameters nor systemic leucocyte count was affected by any of the antibodies employed (data not shown).

**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).

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), beta 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), beta 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 beta 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 HUVEC in a way that reproduces the rolling and adhesion processes that precede the formation of an inflammatory focus.39,40 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)12,19,20 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.24 Lopinavir (10–25 μM in our experiments; 7–17 μM in patients),21 a PI, had no
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 $\beta_2$ 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 $\beta_2$ 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 $\beta_2$ integrins also mediate leucocyte rolling in close dependence on their conformational state.45,46

$\beta_2$ Integrins (CD18) are cell adhesion receptors that share the same CD18 $\beta$ chain but exhibit a distinctive $\alpha$ chain. The family includes four known types: $\alpha_L$ (CD11a, LFA-1), $\alpha_M$ (CD11b, Mac-1), $\alpha_X$ (CD11c, gp95,150) and $\alpha_D$ (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.42 Their main ligand is ICAM-1, which is constitutively expressed on the surface of the vascular endothelium.42 CD11c has already been implicated in phagocytosis, antigen presentation by dendritic cells and the inflammatory response.34 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 thrombosis by mediating the engagement of platelets.48

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 achieved with efavirenz.7,64 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.

**Figure 3.** Leucocyte infiltration in the mesentery of rats treated with efavirenz or nevirapine. Animals were treated (ip) with efavirenz (15 $\mu$M), nevirapine (25 $\mu$M), control (saline) or the corresponding vehicles (efavirenz in methanol; nevirapine in azide water pH $\leq$ 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 $2.5 \times 10^{-4}$ cm$^2$ in animals treated with methanol (a), 15 $\mu$M efavirenz (b), azide water (c) and 25 $\mu$M nevirapine (d). Arrows denote examples of infiltrated neutrophils. Bar = 50 $\mu$m. 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{42}

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{59} differs from that induced by efavirenz in our experiments, 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.

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**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**

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