Low-density lipoprotein and ritonavir: an interaction between antiretrovirals and lipids mediated by P-glycoprotein

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Background: Antiretroviral therapy has considerably reduced HIV disease progression, but complete eradication of HIV cannot actually be achieved. Moreover, prolonged use of protease inhibitors (PIs) causes profound changes in lipid metabolism with an increased risk of cardiovascular diseases. P-glycoprotein (P-gp) is expressed on many cell types, playing an important role in the efflux of drugs including PIs, limiting their intracellular concentration. Furthermore, several studies showed that P-gp is involved in lipid homeostasis and its activity is regulated by cholesterol.

Methods: THP-1 monocytes were used to study: (i) the influence of low-density lipoprotein (LDL) on P-gp expression and function, assessed by flow cytometry and quantitative real-time PCR analysis and measuring ritonavir and rhodamine-123 dye efflux, respectively; and (ii) the influence of ritonavir on cholesterol mobilization. The intracellular levels of ritonavir or cholesterol were measured by HPLC-UV and filipin staining, respectively.

Results: In THP-1 cells, LDL was able to yield an increase in both P-gp expression and activity. THP-1 cells treated with LDL showed a decrease in the intracellular ritonavir concentration in a dose-dependent manner. Notably, ritonavir induced reduced cholesterol mobilization in THP-1 cells, probably due to its inhibitory action on P-gp activity.

Conclusions: Our data indicate a potential interplay between LDL and ritonavir mediated by P-gp. This interaction could influence both therapy effectiveness and cellular lipid metabolism, with important implications in the management of HIV patients treated with boosted PIs.

Keywords: multidrug resistance, protease inhibitors, THP-1

Introduction

The introduction of highly active antiretroviral therapy (HAART) has resulted in a significant reduction in the morbidity and mortality of people infected with HIV.1 Despite suppressing viraemia, HAART cannot fully eradicate the virus due to a persistence of viral reservoirs and ‘sanctuary sites’ not fully affected by the therapies.2 Thus, HIV infection has become a chronic disease, often associated with multiple metabolic abnormalities. In particular, HIV infection is associated with profound changes in lipid metabolism (dyslipidaemia and lipodystrophy), which leads to an increased risk of coronary artery disease.3,4

HAART has been found to produce different effects on lipid metabolism. It has a positive but short-term effect in reversing HIV-related lipid abnormalities soon after the beginning of treatment, followed by long-term negative effects on the lipid profile, with an overall increase in the cardiovascular risk. In particular, ritonavir-boosted protease inhibitors (PIs) are known to increase total cholesterol and low-density lipoprotein (LDL) levels to a greater extent than do non-nucleoside reverse transcriptase inhibitors, which are known to increase high-density lipoprotein (HDL) cholesterol levels markedly.5,6 However, the observed increased risk of myocardial infarction associated with the use of PIs was not fully explained by the plasma lipid changes induced by the
drugs in this class.6,7 Thus, the full mechanism by which PIs may lead to increased rates of myocardial infarction remains to be elucidated. Understanding the differences between antiretroviral drugs with regard to lipid alterations, acceleration of atherosclerosis and cardiovascular risk is crucial to plan regimens that are to be maintained for decades.

P-glycoprotein (P-gp), known as multidrug resistance protein 1 (MDR1) or ATP-binding cassette subfamily B member 1 (ABCB1), is a plasma membrane-located glycoprotein that has the ability to alter the permeability of biological membranes to xenobiotics that may be potentially toxic.8

P-gp modulates the distribution of its substrates, such as chemotherapeutics and antiretroviral drugs, and when overexpressed it may confer multidrug resistance (MDR) by actively excreting drugs from cells, leading to failure of treatment.9,10 In particular, it has been shown that P-gp activity may limit the intracellular accumulation of PIs within cells that support HIV replication.10–13 P-gp is mainly localized within membrane microdomains, termed lipid rafts and caveolae, characterized by a relative rigidity and reduced fluidity, suggesting that membranous cholesterol content may be a modulator of P-gp.14,15 A reciprocal influence between P-gp and cholesterol has been reported. In particular, cholesterol has been shown to affect P-gp activity via drug membrane partitioning and changes to the local environment of the protein.16 Moreover, P-gp expression and activity were affected by cholesterol in ex vivo human peripheral blood mononuclear cells (PBMCs), a feature particularly important for the pharmacokinetics of antiretroviral drugs.17,18

On the other hand, cholesterol efflux can be mediated or regulated by several molecular pathways, including the ATP-binding cassette transporter family, in particular ABCA1, ABCG1 and ABCB1 (P-gp), which plays an important role in lipid elimination from cells.19

The aim of this study was to investigate the effect of cholesterol supplied to cells in the form of LDL on the expression and function of P-gp. The THP-1 monocytic cell line was used to assess whether LDL can modulate the intracellular concentrations of the PI ritonavir and, at the same time, whether ritonavir could influence cholesterol efflux through its action on P-gp.

Methods

Cell line

THP-1, a human monocytic leukaemia cell line, was chosen for this study because it is a highly differentiated monocytic cell line and the presence of scavenger and LDL receptors on THP-1 cells have been reported.20 THP-1 cells were maintained in suspension in 25 cm2 flasks at a cell density of 105 to 106 cells/mL (10 mL) in growth medium containing RPMI-1640 containing 10% fetal bovine serum, 2 mM L-glutamine, 50 µM penicillin and 50 µg/mL streptomycin at 37°C in 5% CO2. Complete medium was replaced twice a week. THP-1 cells were kindly provided by Dr Shary El Daker (Laboratory of Cellular Immunology, National Institute for Infectious Diseases, Lazzaro Spallanzani, Rome, Italy).

Reagents

Human LDL (AppliChem, Milan, Italy) was dissolved in saline solution at a stock concentration of 2 mg/mL and stored at −20°C until use. Ritonavir was purchased from the National Institutes of Health (NIH AIDS Research Reagent Program, Division of AIDS, NIAID, Bethesda, MD, USA), dissolved in DMSO at a stock concentration of 1 mg/mL and stored at +4°C. The doses used in in vitro experiments were 5, 10 and 15 µg/mL, according to maximum non-toxic in vitro concentrations.21 Verapamil (Sigma–Aldrich, St Louis, MO, USA), a P-gp inhibitor, was dissolved in H2O at a stock concentration of 500 µg/mL, stored at −20°C and used at 30 µg/mL.22 Rhodamine-123 [2-[(6-amino-3-imino-3H-xanthen-9-yl)] (Rho; Sigma–Aldrich) is a fluorescent dye. It is a substrate for P-gp and can therefore be used as a molecular probe to assess the activity of P-gp in studies regarding the MDR phenotype.23 Rho was dissolved in DMSO at a stock concentration of 100 µg/mL, stored at +4°C and used at a concentration of 1.5 µg/mL. Filipin III from Streptomyces filipinensis (Sigma–Aldrich) has a specific use for the staining of cholesterol with which it forms a complex (F-C) measurable by specific fluorescent emission.24 Filipin was dissolved in DMSO (1 mg/mL) to make a stock solution, stored at −20°C and used at a concentration of 1.3 µg/mL.

P-gp surface expression by flow cytometry

THP-1 cells or PBMCs (see Supplementary data available at JAC Online for PBMCs) were treated with different concentrations of LDL to assess their effects on P-gp expression. Briefly, cells were cultured in complete medium, without antibiotics in order to avoid possible interference in the activity of P-gp, in a 24-well plate at a density of 1×106/mL and treated with LDL at 50, 100 and 200 µg/mL. After 24 h of incubation at 37°C in 5% CO2, the cells were centrifuged at 1700 rpm for 5 min and incubated with phycerythrin-conjugated anti-P-gp, clone UIC2 antibody (AB Serotec, Oxford, UK) recommended in the consensus guidelines25 for 10 min at 4°C and washed with 200 µL of a washing solution containing 1% PBS, 1% BSA and 0.1% NaN3. Cells were fixed with 100 µL of 1% paraformaldehyde (PFA) for 5 min at room temperature and analysed by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA).26 Fluorescence was detected with fluorescence channel 2 and data were analysed using the Cell Quest program.

Quantification of MDR1 mRNA

THP-1 cells were treated with different concentrations of LDL as described above. After different incubation times (1, 6, 12 and 24 h), RNA was extracted with Trizol reagent (Invitrogen Life Technologies, Monza, Italy) according to the manufacturer’s instructions. cDNA was generated from 1 µg of RNA using the AMV reverse transcription kit (Promega, Milan, Italy) according to the manufacturer’s recommendations. Real-time PCRs were performed using an RG-600 thermal cycler (Corbett Life Science, Australia). The Maxima SYBR Green qPCR Master Mix kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to produce fluorescently labelled PCR products during repetitive cycling of the amplification reaction (95°C for 10 min followed by 40 cycles of 95°C for 10 s, 60°C for 30 s and 72°C for 30 s). Fluorescence values were analysed using LightCycler software version 3.5 and relative amounts were obtained using the 2−ΔΔCT method and normalized to L34 gene expression. The following primer sets were used: P-gp (MDR1) forward 5′-AACACAGCATTCGCTCA GTCCTG-3′ and P-gp (MDR1) reverse: 5′-AGTCTGACTCTGAGTGTGGTGC-3′; and L34 forward 5′-GTCCGAAACCCTGTTAAG-3′ and L34 reverse 5′-GGCTCTGCTGACATGGTTCT-3′.

Evaluation of P-gp functional activity using Rho

THP-1 cells or PBMCs were adjusted to 1×106 cells/mL (10 mL) in growth medium without antibiotics, treated with Rho (1.5 µg/mL) and incubated at 4°C for 25 min; cells without Rho (1×106 cells/mL; 1 mL) were used as a negative control. After incubation, cells were washed twice, resuspended in complete medium, divided into aliquots (1×106/mL) and stimulated with increasing concentrations of LDL (0, 50, 100 and 200 µg/mL) for...
Quantification of ritonavir intracellular concentration

THP-1 cells were suspended in medium without antibiotics (1 x 10^5/mL; 50 mL) and divided into aliquots (10 mL). To the aliquots was added ritonavir (5 μg/mL), either alone (negative control) or with different amounts of LDL (50, 100 and 200 μg/mL), for 24 h. An aliquot containing ritonavir, LDL (200 μg/mL) and verapamil (30 μg/mL) was considered as a positive control of P-gp inhibition. After incubation, THP-1 cells were washed in ice-cold PBS and centrifuged at 1700 rpm for 5 min. The resulting pellet was dissolved with 1 mL of extraction solution (methanol/water; 70:30, v/v), centrifuged at 12,000 rpm and the supernatants were evaporated to dryness. Dried THP-1 extracts were reconstituted in 100 μL of a solution consisting of H2O and acetonitrile (60:40, v/v) for subsequent HPLC-UV analysis with a validated method.27,28 The ritonavir concentration in THP-1 cells was normalized versus the control considering the aliquot treated with ritonavir as 100%.

Quantification of intracellular cholesterol concentration

In order to quantify the intracellular cholesterol concentration, THP-1 cells were cultured with LDL (200 μg/mL), ritonavir (5, 10 and 15 μg/mL) and verapamil (30 μg/mL) for 24 h. For detection of F-C complexes, cells in culture were washed twice, resuspended in 600 μL of PBS and for all the experimental conditions we made two aliquots: one aliquot was used as a ‘blank’ and the other aliquot was labelled with filipin at a final concentration of 1.3 μg/mL for 1 h at room temperature. After incubation, cells were washed twice with 1 mL of cold PBS, centrifuged, resuspended in 300 μL of PBS and F-C complexes were visualized by using a Victor fluorometer with excitation at 330–385 nm (emission at 420 nm).24

Statistical analysis

Statistical analysis was performed using the GraphPad Prism package, version 5.02 (GraphPad Software, San Diego, CA, USA). When comparing differences between groups, Wilcoxon and Mann-Whitney non-parametric tests were used. A P value <0.05 was considered statistically significant.

Results

LDL increased expression of P-gp

To assess whether LDL can influence P-gp expression, THP-1 cells were treated for 24 h with different concentrations of lipoproteins. P-gp surface expression was evaluated by flow cytometry.

Figure 1 shows the results and statistical analysis obtained from 13 experiments.

A very low percentage of P-gp-positive cells was found in untreated THP-1 cells (1.20% ± 0.64%), while treatment with 50, 100 and 200 μg/mL LDL increased P-gp expression to 2.54% ± 1.14%, (P<0.01), 5.52% ± 1.51% (P<0.001) and 9.33% ± 3.88% (P<0.001), respectively.

The same experiment was conducted in ex vivo monocytes to evaluate LDL effects on primary cells. Preliminary results using PBMCs from eight healthy donors showed that the incubation with LDL significantly (P<0.001) increased P-gp expression on the primary monocytes (see the Supplementary data available at JAC Online).

Since exposure of P-gp on the plasma membrane surface could be due to either cellular redistribution or new synthesis, we measured MDR1 gene expression upon LDL stimulation at different timepoints. As shown in Figure 2, LDL induced a significant increase in MDR1 mRNA levels after 6 h of treatment, which decreased to basal levels after a further 6 h. Since: (i) there were no significant differences between the MDR1 mRNA levels of cells stimulated with 50 or 100 μg/mL, while their P-gp surface expression is different; and (ii) the surface P-gp localization was measured 24 h after the MDR1 mRNA levels, we can conclude that LDL are able to both induce MDR1 gene expression and protein trafficking.

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**Figure 1.** LDL treatment increases P-gp expression. THP-1 cells incubated for 24 h with increased concentrations of LDL. The box encompasses the IQR of individual measurements and the horizontal bar in the box indicates the median value of 13 experiments. Data were analysed using the Mann-Whitney U-test: **P<0.01 and ***P<0.001.

**Figure 2.** MDR1 mRNA increases after LDL treatment. Levels of MDR1 mRNA were measured in THP-1 cells either untreated or treated with the indicated doses of LDL by qPCR. qPCR values were normalized to L34 mRNA levels and reported as fold changes relative to control samples (mean± SD of three experiments).
Levels of MDR1 mRNA were measured in THP-1 cells either untreated or treated with the indicated doses of LDL by quantitative real-time PCR (qPCR). qPCR values were normalized to L34 mRNA levels and reported as fold changes relative to control samples (mean ± SD of three experiments).

**LDL increased Rho efflux**

To investigate whether treatment with LDL can influence P-gp activity, we measured the efflux of Rho. THP-1 cells were stained, incubated with increasing concentrations of LDL (0, 50, 100 and 200 μg/mL) and Rho intracellular concentrations measured as mean fluorescence intensity (MFI) by flow cytometry. Figure 3 shows the statistical results of eight experiments.

Treatment with different concentrations of LDL induced an increase in Rho efflux in THP-1 cells in a dose-dependent fashion with respect to untreated cells (considered as 100%). THP-1 cells treated with Rho and 50, 100 and 200 μg/mL LDL showed an MFI of 95% ± 4% (P < 0.05), 90% ± 4% (P < 0.05) and 82% ± 2% (P < 0.05), respectively, in comparison with untreated control cells.

In contrast, verapamil (30 μg/mL) added with 200 μg/mL LDL blocked P-gp activity compared with cells treated only with 200 μg/mL LDL (P < 0.05).

Ex vivo experiments showed that LDL also increased P-gp functionality in primary cells from healthy donors. Indeed, monocytes had a significant (P < 0.001) decrease in levels of Rho after LDL incubation. The intracellular Rho efflux showed an increase depending on LDL concentration (see the Supplementary data available at JAC Online).

**LDL increased ritonavir efflux in vitro**

To find out if treatment with LDL was able to alter the intracellular concentration of ritonavir, we carried out a quantitative analysis of ritonavir concentrations inside the cells by means of the HPLC-UV method. Briefly, THP-1 cells were treated for 24 h at 37°C with different concentrations of LDL and with a constant concentration of ritonavir (5 μg/mL). THP-1 cells treated with ritonavir alone were used as a control for intracellular drug accumulation.

As shown in Figure 4, treatment with LDL was able to decrease significantly in a dose-dependent manner the intracellular concentration of ritonavir in THP-1 cells with respect to the control. The percentage of retention of ritonavir in cells treated with 50, 100 and 200 μg/mL LDL was 83.5% ± 4% (not significant), 76.5% ± 19% (P < 0.01) and 63% ± 30% (P < 0.01), respectively, compared with the amount of ritonavir in the cells not treated with LDL.

In contrast, treatment with verapamil was able to block P-gp activity, showing a ritonavir intracellular concentration similar to the control.

**Ritonavir increased intracellular cholesterol**

The effect of inhibitors of P-gp (ritonavir and verapamil) on the level of cholesterol in THP-1 cells was investigated. THP-1 cells were treated with 200 μg/mL LDL in the absence or presence of different concentrations of ritonavir, and F-C complexes, measured as MFI, were evaluated. The value obtained in cells treated with 200 μg/mL LDL alone was regarded as unit value 1, to which the other experimental conditions were normalized.

As shown in Figure 5, treatment with ritonavir was able to increase in a dose-dependent manner the intracellular concentration of cholesterol in THP-1 cells with respect to the control.

In THP-1 cells treated with LDL and 5 μg/mL ritonavir the mean intracellular cholesterol ratio is equal to 1.02 ± 0.14 (with respect to the untreated control), in cells treated with LDL and 10 μg/mL ritonavir the average intracellular cholesterol ratio is equal to 1.63 ± 0.32, in cells treated with LDL and 15 μg/mL ritonavir the average intracellular cholesterol ratio is equal to 1.83 ± 0.5.
Ex vivo experiments were conducted in monocytes obtained from healthy donors. Our data demonstrate that THP-1 cells do not constitutively express P-gp and that treatment with LDL increases the expression of P-gp on the cellular surface, probably due to both gene transcription and protein trafficking. Notably, this P-gp expression causes an enhancement in its substrate efflux activity. In fact, LDL increased the efflux potential of either the fluorescent dye Rho or ritonavir, a typical antiretroviral drug substrate and inhibitor of P-gp usually used as a booster for other PIs. We proved that intracellular concentrations of Rho and ritonavir decrease inversely to the concentration of LDL. The preliminary data, obtained in ex vivo PBMCs, confirm that LDL increases P-gp expression and functionality also in primary monocytes, increasing the clinical relevance of our results.

It is tempting to speculate that the LDL-induced P-gp up-regulation is mediated by cholesterol. In fact, it could modify membrane microdomains that are rich in lipids redistributing from the inner leaflet of the membrane to the outer leaflet. Moreover, the data obtained by Troost et al., who described an increased P-gp functionality in lymphocytes and monocytes treated with cholesterol (5-cholesten-3β-ol) and methyl-β-cyclodextrin, confirm this hypothesis. P-gp is at least partially located in cholesterol- and sphingolipid-enriched parts of the plasma membrane called “lipid rafts”. The association of high P-gp content in membranous regions with lower fluidity suggests that membranous cholesterol content may be a modulator of P-gp function. It has been demonstrated that cholesterol depletion of the plasma membrane inhibits Rho transport. Therefore, in our cellular model it is likely that the increased uptake of cellular cholesterol mediated by LDL can alter the lipid content of membranes, which regulates P-gp expression and activity.

The mechanism that we describe could explain the beneficial effect of LDL apheresis in some clinical cases of nephrotic syndrome associated with a high level of LDL and resistance to steroid and cyclosporine therapy. Indeed, Ueda et al. reported an enhanced MDR1 expression at the time of recurrence in patients with steroid-resistant nephrotic syndrome, which was restored after a course of LDL apheresis therapy by reducing the level of MDR1 and therefore the activity of P-gp. It is noteworthy that cyclosporine is a recognized substrate for P-gp (as ritonavir) whose pharmacological efficacy and distribution is influenced by P-gp expression and functionality. Based on these results, the authors concluded that LDL adsorption therapy decreased MDR1 expression sharply, restoring drug susceptibility for immunosuppressants.

Therefore, it is tempting to speculate on a possible cascade of events that can lead to MDR in HIV therapy: antiretroviral treatment, particularly with boosted PIs, increases plasma levels of total cholesterol and LDL cholesterol, thus increasing P-gp-mediated efflux activity and not permitting therapeutic concentrations of drugs inside cells to be achieved.

In addition to drugs, P-gp extrudes numerous physiological substrates or metabolites such as phospholipids and sex-steroid hormones and several facts support a role for P-gp in cholesterol homeostasis. Several studies demonstrated that ritonavir has a significant impact on the expression of master regulators of cholesterol trafficking in human macrophages, increasing its intracellular concentration. Wang et al. reported that, in THP-1-derived foam cells, ritonavir markedly reduced cholesterol efflux in response to both apoA-1 and HDL. Moreover, Dressman et al. also reported that ritonavir could increase the level of cholesterol ester in THP-1 cells.

**Figure 5.** Ritonavir decreases intracellular cholesterol. In all conditions, THP-1 cells were incubated with a single dose of LDL (200 μg/mL). Cells treated with LDL alone were considered as the control for cholesterol accumulation (black bar). THP-1 cells in the presence of increasing concentrations of ritonavir (white bars) and ritonavir 15 μg/mL plus verapamil (grey bar) represent the fold increase in the intracellular concentration of cholesterol with respect to the control. Data are expressed as the mean ± SEM of six different experiments. Statistical analysis was performed using the Wilcoxon test: *P<0.05.

In cells stimulated with LDL and verapamil, we observed the maximum intracellular cholesterol ratio (2.45 ± 1.24, with respect to the untreated control). When P-gp is inhibited by drugs, cholesterol, introduced by LDL, accumulates in THP-1 cells. It would seem that the more the drug is capable of inhibiting P-gp, the greater the concentration of cholesterol that remains in the cells.

**Discussion**

HAART has transformed HIV infection from an acute illness to a manageable chronic disease, but two important points have emerged associated with long-term HAART treatment containing PIs: Firstly, the appearance of several clinical and metabolic complications; PI treatment may be associated with an increased risk of cardiovascular diseases due to atherosclerosis along with metabolic syndromes including systemic insulin resistance, dyslipidemia and peripheral lipodystrophy. Moreover, PI-associated dyslipidemia is characterized by hypertriglyceridaemia with depressed plasma concentrations of HDL cholesterol, increased total cholesterol and increased LDL cholesterol. Secondly, the existence of sanctuary sites such as resting latently infected CD4+ cells and monocytes, which are the major targets of HIV-1 infection, prevent complete eradication of the virus. It is thought that MDR transporters may play a role in lowering intracellular drug concentrations at these sites via an efflux mechanism.

The aim of this study was to understand whether there exists a common way of interfering with cellular functional pathways by antiretroviral drugs and LDL. For this purpose the effect on P-gp, an efflux pump that extrudes a wide variety of lipophilic molecules from cells including many antiretrovirals and lipids, was evaluated.

We employed highly differentiated monocytic cells (THP-1) in order to reproduce the potential behaviour of primary monocytes, increasing the clinical relevance of our results. We proved that intracellular concentrations of Rho and ritonavir decrease inversely to the concentration of LDL. The preliminary data, obtained in ex vivo PBMCs, confirm that LDL increases P-gp expression and functionality also in primary monocytes, increasing the clinical relevance of our results.

It is tempting to speculate that the LDL-induced P-gp up-regulation is mediated by cholesterol. In fact, it could modify membrane microdomains that are rich in lipids redistributing from the inner leaflet of the membrane to the outer leaflet. Moreover, the data obtained by Troost et al., who described an increased P-gp functionality in lymphocytes and monocytes treated with cholesterol (5-cholesten-3β-ol) and methyl-β-cyclodextrin, confirm this hypothesis. P-gp is at least partially located in cholesterol- and sphingolipid-enriched parts of the plasma membrane called “lipid rafts”. The association of high P-gp content in membranous regions with lower fluidity suggests that membranous cholesterol content may be a modulator of P-gp function. It has been demonstrated that cholesterol depletion of the plasma membrane inhibits Rho transport. Therefore, in our cellular model it is likely that the increased uptake of cellular cholesterol mediated by LDL can alter the lipid content of membranes, which regulates P-gp expression and activity.

The mechanism that we describe could explain the beneficial effect of LDL apheresis in some clinical cases of nephrotic syndrome associated with a high level of LDL and resistance to steroid and cyclosporine therapy. Indeed, Ueda et al. reported an enhanced MDR1 expression at the time of recurrence in patients with steroid-resistant nephrotic syndrome, which was restored after a course of LDL apheresis therapy by reducing the level of MDR1 and therefore the activity of P-gp. It is noteworthy that cyclosporine is a recognized substrate for P-gp (as ritonavir) whose pharmacological efficacy and distribution is influenced by P-gp expression and functionality. Based on these results, the authors concluded that LDL adsorption therapy decreased MDR1 expression sharply, restoring drug susceptibility for immunosuppressants.

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In addition to drugs, P-gp extrudes numerous physiological substrates or metabolites such as phospholipids and sex-steroid hormones and several facts support a role for P-gp in cholesterol homeostasis. Several studies demonstrated that ritonavir has a significant impact on the expression of master regulators of cholesterol trafficking in human macrophages, increasing its intracellular concentration. Wang et al. reported that, in THP-1-derived foam cells, ritonavir markedly reduced cholesterol efflux in response to both apoA-1 and HDL. Moreover, Dressman et al. also reported that ritonavir could increase the level of cholesterol ester in THP-1 cells.
macrophages and human PBMCs. In these studies, the possibility has not been evaluated that P-gp may contribute to the decrease of cholesterol efflux.

Our results show an increased cholesterol intracellular concentration in THP-1 cells when high concentrations of ritonavir were present in the culture medium. This effect seems to be caused by inhibition of P-gp, since treatment of the cells with verapamil, a potent P-gp inhibitor, also induced an increase in intracellular cholesterol. Ritonavir may contribute to the accumulation of cholesterol in monocytes/macrophages by inhibiting cholesterol efflux, which most likely could play a part in the progression of atherosclerosis.

Since it was found that intracellular concentrations of ritonavir are influenced by PIs, the results here reported may also be important in driving clinicians to choose the best therapy from the point of view of intracellular cholesterol modulation. This opens new perspectives in the use of drugs such as ritonavir in antiretroviral therapy. In fact, the use of ritonavir can induce an increase of circulating LDL and, through the inhibition of P-gp, can promote the accumulation of intracellular cholesterol, the formation of foam cells and the progression to lesions characteristic of atherosclerosis, suggesting a possible mechanism for the increased cardiovascular risk described in HIV-positive patients on antiretroviral therapy.

We have shown the existence at the cellular level of interactions between ritonavir and LDL, but further data on other antiretrovirals are needed. Ritonavir intracellular levels may not represent a crucial issue in determining the effectiveness of a PI-containing HAART since, theoretically, its antiviral activity seems to be related to the ‘boosting’ effect on the main PI plasma levels. However, since ritonavir has been found at high concentrations intracellularly, it has been started to be assumed that it has a synergistic antiviral effect with the accompanying PI. Moreover, lipid changes may be relevant in triggering and progressing atherosclerosis in treated HIV patients.

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Transparency declarations
None to declare.

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

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