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Estrogen Prevents Cholesteryl Ester Accumulation in Macrophages Induced by the HIV Protease Inhibitor Ritonavir

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Abstract Individuals with HIV can now live long lives with drug therapy that often includes protease inhibitors such as ritonavir. Many patients, however, develop negative long-term side effects such as premature atherosclerosis. We have previously demonstrated that ritonavir treatment increases atherosclerotic lesion formation in male mice to a greater extent than in female mice. Furthermore, peripheral blood monocytes isolated from ritonavir-treated females had less cholesteryl ester accumulation. In the present study, we have investigated the molecular mechanisms by which female hormones influence cholesterol metabolism in macrophages in response to the HIV protease inhibitor ritonavir. We have utilized the human monocyte cell line, THP-1 as a model to address this question. Briefly, cells were differentiated for 72 h with 100 nM PMA to obtain a macrophage-like phenotype in the presence or absence of 1 nM 17β-estradiol (E2), 100 nM progesterone or vehicle (0.01% ethanol). Cells were then treated with 30 ng/ml ritonavir or vehicle in the presence of aggregated LDL for 24 h. Cell extracts were harvested, and lipid or total RNA was isolated. E2 decreased the accumulation of cholesteryl esters in macrophages following ritonavir treatment. Ritonavir increased the expression of the scavenger receptor, CD36 mRNA, responsible for the uptake of LDL. Additionally, ritonavir treatment selectively increased the relative levels of PPARγ mRNA, a transcription factor responsible for the regulation of CD36 mRNA expression. Treatment with E2, however, failed to prevent these increases at the mRNA level. E2 did, however, significantly suppress CD36 protein levels as measured by fluorescent immunocytochemistry. This data suggests that E2 modifies the expression of CD36 at the level of protein expression in monocyte-derived macrophages resulting in reduced cholesteryl ester accumulation following ritonavir treatment. J. Cell. Biochem. 103: 1598–1606, 2008.

Key words: CD36; PPARγ; atherosclerosis; macrophage; ritonavir; estrogen; steroid

Highly active anti-retroviral therapy (HAART) has significantly lengthened the lifespan of individuals infected with the human immunodeficiency virus (HIV). Blocking the processing of HIV with protease inhibitors included in HAART delays or prevents the onset of AIDS. Unfortunately, one of the deleterious side effects of protease inhibitor therapy is the potential development of premature atherosclerosis [Currier et al., 2003], particularly in younger patients [Mooser, 2003]. HIV protease inhibitor treatment also causes dyslipidemia that may contribute to the development of cardiovascular disease [Carr et al., 1998]. Macrophage recruitment into the vascular wall is an early event in the development of atherosclerosis, and the uptake of lipoprotein by macrophages in the sub-endothelial space contributes to the formation of lipid-laden macrophages and ultimately fatty streaks. A number of different macrophage cell surface receptors for lipoproteins have been identified [Acton et al., 1994]. The interplay between these receptors, lipoproteins, and cellular cholesterol metabolism can regulate the extent of lipid accumulation in macrophages. The scavenger receptor, CD36, has been shown to mediate both the uptake and efflux of cholesterol

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from cells playing a critical role in the development of atherosclerosis [Nicholson, 2004] and its up-regulation is responsible for cholesteryl ester accumulation induced by ritonavir [Dressman et al., 2003]. Of all the scavenger receptors expressed in macrophages, CD36 mRNA and protein levels are specifically increased by ritonavir treatment. Furthermore, preventing this increase by molecular manipulation reverses the accumulation of cholesteryl esters induced by ritonavir.

Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors activated by many naturally occurring fatty acid derivatives. Three isoforms have been identified (PPARα, PPARγ, PPARδ) that are encoded by three separate genes [Berger and Moller, 2002]. In the nucleus, activated PPARs heterodimerize with the retinoid X receptor (RXR) and bind to peroxisome proliferator response elements (PPREs) in the promoters of target genes. Stimulation of both PPARγ and PPARα results in the increase of CD36 mRNA expression [Tontonoz et al., 1998; Sato et al., 2002]. This PPARγ-CD36 stimulatory loop serves to increase the uptake of oxidized LDL. Additionally, PPARγ activation can lead to the up-regulation of the ABCA1 cholesterol transporter [Akiyama et al., 2002], suggesting that PPARγ also stimulates the efflux of cholesterol. Transcription of ABCA1 is mediated by LXRα, which in turn, is stimulated by PPARγ [Lee and Evans, 2002]. Together, these observations indicate PPARγ plays a central role in lipid metabolism. While multiple mechanisms are involved in modified lipid uptake, we have previously demonstrated that PPARγ-mediated up-regulation of CD36 is required for ritonavir-induced accumulation of cholesteryl esters [Dressman et al., 2003].

Gender differences in atherosclerosis are well known. In general, females have a decreased risk of developing cardiovascular disease prior to menopause as compared to men. Even in light of the Women's Health Initiative that indicated current hormone replacement therapy is detrimental to cardiovascular health in older postmenopausal women [Rossouw et al., 2002], female sex hormones have been shown to have numerous positive effects on the cardiovascular system (reviewed in Mendelsohn and Karas [2005]). The reasons for these differences are a current focus of intensive investigation. We have previously observed gender differences in the development of atherosclerosis induced by HIV protease inhibitors in a mouse model [Allred et al., 2006]. Female mice developed significantly fewer lesions than male mice. Furthermore, we determined that the site of this effect is at the level of the macrophage as peritoneal macrophages isolated from female mice lacking the estrogen receptor accumulate cholesteryl esters equivalent to levels of those from males. This effect is independent of alterations in serum cholesterol levels. In the present study, we have begun to investigate some of the molecular events involved in the regulation of the factors that mediate the effects of estrogen on cholesteryl ester accumulation in macrophages in response to the HIV protease inhibitor, ritonavir.

EXPERIMENTAL METHODS

Cell Culture

The human monocyte cell line, THP-1 (ATCC), was grown in phenol red-free RPMI 1640 medium containing 10% charcoal-dextran-stripped bovine calf serum, 100 U/ml penicillin-streptomycin, and 2 mM L-glutamine. Eighty percentage of the media was changed every 2–3 days. THP-1 cells were grown in the presence of 100 nM PMA for 72 h to induce differentiation to a macrophage phenotype. Aggregated LDL (agLDL; 50 μg/ml) was added to the culture medium as the source of exogenous modified LDL. agLDL was prepared by diluting 2 mg freshly isolated LDL in 1 ml PBS and vortexing for 60 s. One hundred nanomolars stock 17β-estradiol (E2) was dissolved in 1% ethanol and added to media in a 1:100 dilution for a final concentration of 1 nM E2 and 0.01% ethanol.

Cholesterol and Cholesteryl Ester Mass Quantification

Cholesterol mass was determined as previously described [Uittenbogaard et al., 2002]. Briefly, the extracted dried total cellular lipid was derivatized by suspension in N,O-bis(trimethylsilyl)trifluoroacetamide, trimethylchlorosilane, and acetonitrile (89:1:10). The material was heated to 80°C for 5 min, dried, suspended in iso-octane, and subjected to gas chromatography (Protocol T496125B, Supelco). Pure cholesterol (Sigma–Aldrich) was used as a standard for the retention time of cholesterol. The samples were injected into a 6890 gas

chromatograph G2579A system (Agilent Technologies, Palo Alto, CA) equipped with a SGE HT5 aluminum-clad fused silica capillary column (12 m × 0.32 mm × 0.1 μm; Sigma–Aldrich). The gas chromatography temperature program was as follows: initial temperature was 220°C for 3 min, 310°C (20°C increase/min), and then 400°C (10°C/min) for 3.5 min. A mass-selective detector (model 5973; Agilent Technologies) was used in both scan and selected ion-monitoring modes to identify the samples.

RT-PCR

Cells (1×10^6) were lysed in 1 ml of TRIZOL Reagent and RNA was isolated as previously described. The RNA pellet was briefly air dried and suspended in 50 μl RNase-free water and stored at −70°C. One microgram of total RNA for each sample and the appropriate amount of DEPC H_2O was added to bring the total volume to 10 μl. One microliter of random primers (Invitrogen) and 1 μl of 10 mM dNTP's were added to each reaction. The samples were incubated at 65°C for 5 min and then chilled on ice. One microgram of RNA was incubated with a RT-master mix containing 4 μl of 5× first strand buffer, 2 μl 0.1 M DTT, 1 μl RNasin and 1 μl Superscript RT. Samples were then incubated at room temperature for 10 min, 42°C for 50 min and 70°C for 15 min. Real-time PCR was then performed for each sample in triplicate in a 96-well plate. Each reaction contained 21 microliters of DEPC H_2O, 25 μl of 2X SYBR Green Brilliant master mix (Stratagene), 1 μl (10–50 pmol) of upstream primer, 1 μl (10–50 pmol) of downstream primer, 0.75 μl of reference dye (diluted 1:500) (Stratagene) and 1 μl of appropriate cDNA. Primer concentrations were previously optimized for each gene. Each 96-well plate contained non-template control well and all data was normalized to the housekeeping gene Histone 3.1. The primers and annealing temperatures for each gene were previously described (Table I). Cycling parameters were as follows: 1 cycle at 95°C for 10 min followed by 40 cycles of 95°C for 30 s, appropriate annealing temperature for 1 min and 72°C for 30 s. Real-time fluorescent measurements were taken at every cycle and change in threshold cycle (Δ^{C_t}) was calculated. The comparative $\Delta\Delta^{C_t}$ method was used to compare the experimental groups to the control vehicle/vehicle cells [Pfaffl, 2001].

Immunocytochemistry

Immunocytochemistry was performed on cells grown on glass cover slips and fixed in 4% paraformaldehyde. Following a 1 h blocking step at room temperature (PBS, 1% Triton-X, 5% normal donkey serum) the primary antibody incubation (CD36 1:200, Biodesign International P54168M) was carried out at 4°C overnight. Cells were washed in PBS three times and incubated with an anti-rabbit TexasRed-conjugated secondary antibody (Jackson Laboratory, 1:100) for 1 h at room temperature. Controls lacking primary and secondary antibodies were included. Cells were counterstained with DAPI to identify and count nuclei. Images were captured following epifluorescence microscopy using a SpotRT camera/ and software system. Fluorescent intensity was determined using ImagePro Plus (v. 5.1) software. Ten random fields for each sample were captured at a magnification of 200× and overall fluorescence measured. Background from a cell-free area was subtracted. The overall fluorescence was divided by the total number of cells, giving an average intensity per cell. The experimenter performing the measurements was blinded to the groups.

Western Immunoblot Assay

THP-1 cells were differentiated with PMA for 72 h and treated with either vehicle (0.001% ethanol), 1 nM 17β-estradiol, or 10 nM 17β-estradiol for 24 h prior to the addition of 30 ng/ml ritonavir or vehicle. Following 24 h of ritonavir treatment cells were lysed and equal

TABLE I. Primers and Conditions for Real-Time RT-PCR

Gene	Forward primer	Reverse primer	Annealing temp. (°C)
CD36	CCAGACAACTATTGTTTCTGC	ATCACCACACCAACACTGAG	57
ABCA1	GCTGCTGAAGCCAGGGCATGGG	GTGGGGCAGTGGCCATACTCC	60
PPARα	ATCGGCGAGGATAGTTCT	AATCGCGTTGTGTGACAT	57
PPARδ	CAGAAGAAGAACCGCAACA	CGCCATAACTTGAGAAGGGT	60
PPARγ	CAGATCCAGTGGTTGCAG	GTCAGCGGACTCTGGATT	57
LXRα	CTTCCACTACAAGTTGTGAGCTGC	GGCAGCGACGAGCTTCTCGATCAGT	60
RXRα	AACCGCTGCCAGTACTGC	AAAGACCAGGTCGGGAGG	60

amounts of protein were resolved by SDS–PAGE and immunoblotted with the antibody for CD36 (1:500). Actin served as a loading control (data not shown). Representative data from three independent experiments are shown.

Statistics

Data were analyzed by two-way analysis of variance (ANOVA) (Drug X E2), one-way ANOVA, or the Student Newman–Keuls *t*-test for post-hoc comparisons where appropriate. Significance was considered at a $P < 0.05$. All experiments consisted of $n = 3–6$ replicates per experimental group, and each experiment was repeated at least three independent times with similar results.

RESULTS

17β-Estradiol Suppresses Cholesteryl Ester Accumulation in THP-1 Cells

A human monocyte/macrophage cell line, THP-1, was treated with ritonavir to determine if ritonavir induces cholesteryl ester accumulation as it does with mouse peritoneal macrophages. We chose to use the human cell line instead of isolated human macrophages to eliminate the variability from inherent differences from donors. THP-1 cells were differentiated with PMA to obtain a macrophage phenotype. The cells were then incubated with 50 μg of agLDL and 30 ng/ml ritonavir or vehicle (0.01% ethanol) for 24 h. This dose of ritonavir is relatively low, however, it has previously shown that it has significant effects on cholesteryl ester metabolism in peritoneal macrophages as well as THP-1 cells [Dressman et al., 2003]. Low doses of ritonavir induce the development of atherosclerosis without causing the complicating consequences of dyslipidemia. Ritonavir significantly increased the amount of cholesteryl esters accumulated within the cells (Fig. 1A). Pretreatment with 1 nM 17β-estradiol (E2) at the time of differentiation significantly suppressed the amount cholesteryl ester accumulation induced by ritonavir ($P < 0.01$). Pretreatment with 100 nM progesterone (P4) had no effect and co-incubation of E2 and P4 resulted in levels similar to E2 alone. The levels of free cholesterol were not altered by ritonavir or hormone treatments (Fig. 1B), indicating the sterol from agLDL is likely located in esterified compartment.

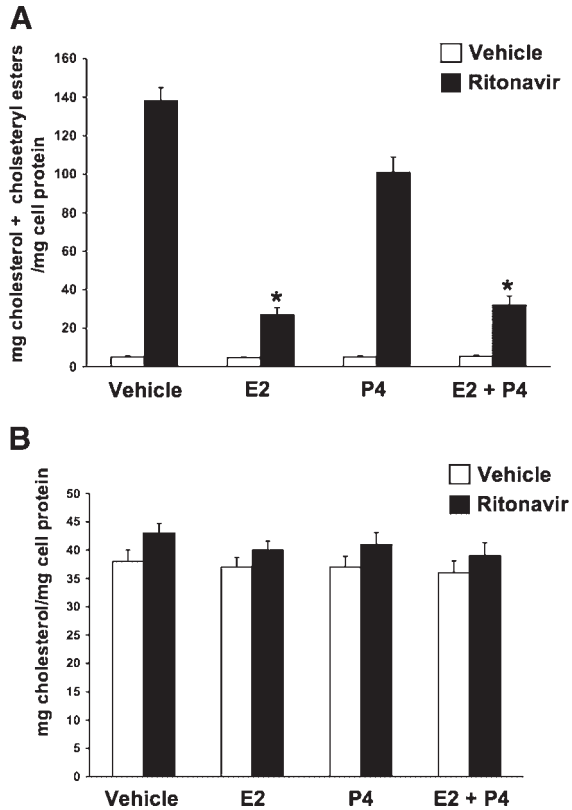

Fig. 1. 17β-estradiol (E2) prevents cholesteryl ester accumulation in THP-1 cells treated with ritonavir. Differentiated THP-1 cells were pretreated with vehicle (0.01% EtOH), 1 nM E2, 100 nM progesterone (P4), or E2 + P4 for 24 h and then incubated with 50 μg/ml of agLDL along with 30 ng/ml ritonavir or vehicle (0.01% ethanol) for an additional 24 h. Cells were lysed, lipids extracted, and processed to quantify cholesteryl esters (**A**) and total cholesterol (**B**) by gas chromatography. Bars represent the mean ± SE, $n = 3$. Asterisk (*) significantly different than vehicle/ritonavir ($P < 0.01$).

17β-Estradiol Does not Have an Effect on CD36 mRNA Levels

The scavenger receptor CD36 has been shown to be responsible for the increased uptake of LDL and accumulation of cholesteryl esters within macrophages induced by ritonavir [Dressman et al., 2003]. In the present study, we determined the effects of ritonavir and estrogen treatment on CD36 mRNA levels. In addition, for completeness we also examined the cholesterol efflux transporter, ATP-binding cassette transporter A1 (ABCA1) and the complement of transcription factors that regulate both genes' expression by semi-quantitative real-time RT-PCR. Cells were treated as described for Figure 1. Ritonavir increased CD36 mRNA levels ($P < 0.05$; Fig. 2A), however, E2 did not alter this increase in CD36 expres-

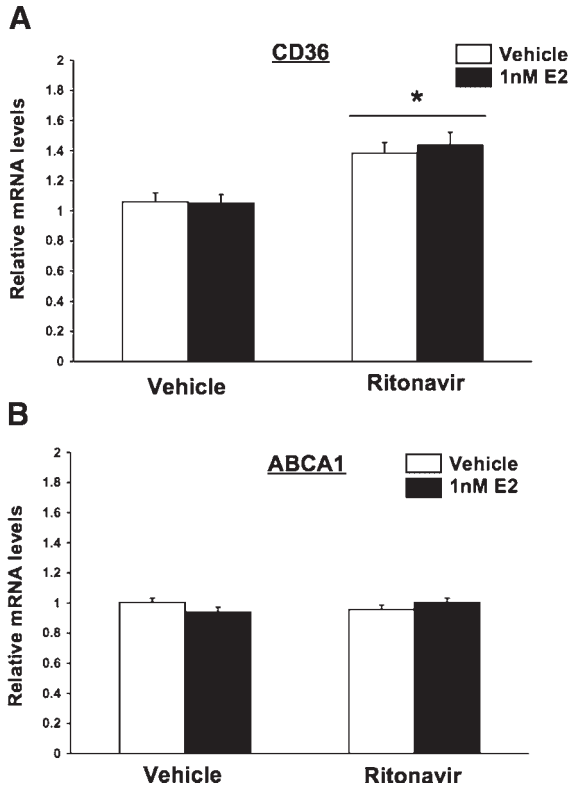

Fig. 2. Ritonavir selectively increases CD36 mRNA expression. Differentiated THP-1 cells were pretreated with vehicle (0.01% EtOH) or 1 nM E2 for 24 h and then incubated with 50 μg/ml of agLDL along with 30 ng/ml ritonavir or vehicle (0.01% ethanol) for 24 h. Cells were lysed, total RNA isolated and real-time RT-PCR performed. All data was normalized to an internal housekeeping control gene and expressed relative to the vehicle/vehicle control group. Bars represent mean ± SEM, n = 3. Asterisk (*) significantly different from vehicle ($P < 0.05$).

sion. Neither ritonavir nor E2 had an effect on ABCA1 mRNA expression.

PPARγ mRNA Levels Are Increased by Ritonavir

The PPAR family of transcription factors plays a role in regulating numerous aspects of cholesterol metabolism in macrophages including increasing CD36 levels. Ritonavir treatment significantly increased only PPARγ ($P < 0.05$) mRNA levels (Fig. 3). Pretreatment with E2 did not alter this increase. Both E2 and ritonavir appear to increase PPARδ mRNA expression, however this did not reach statistical significance. In addition to the PPARs, the nuclear hormone receptors RXRα participate in the regulation of the cholesterol metabolism machinery by forming heterodimers with PPARs and regulating the expression of CD36. Additionally, LXRα is a transcription factor stimulated by PPARγ that increases ABCA1 expression. Ritonavir treatment did not alter the expression of either RXRα or LXRα (data not shown). E2 treatment alone or in combination with ritonavir also had no effect.

17β-Estradiol Prevents the Increase in CD36 Protein Induced by Ritonavir

To confirm that changes in mRNA result in changes in protein levels, we also examined

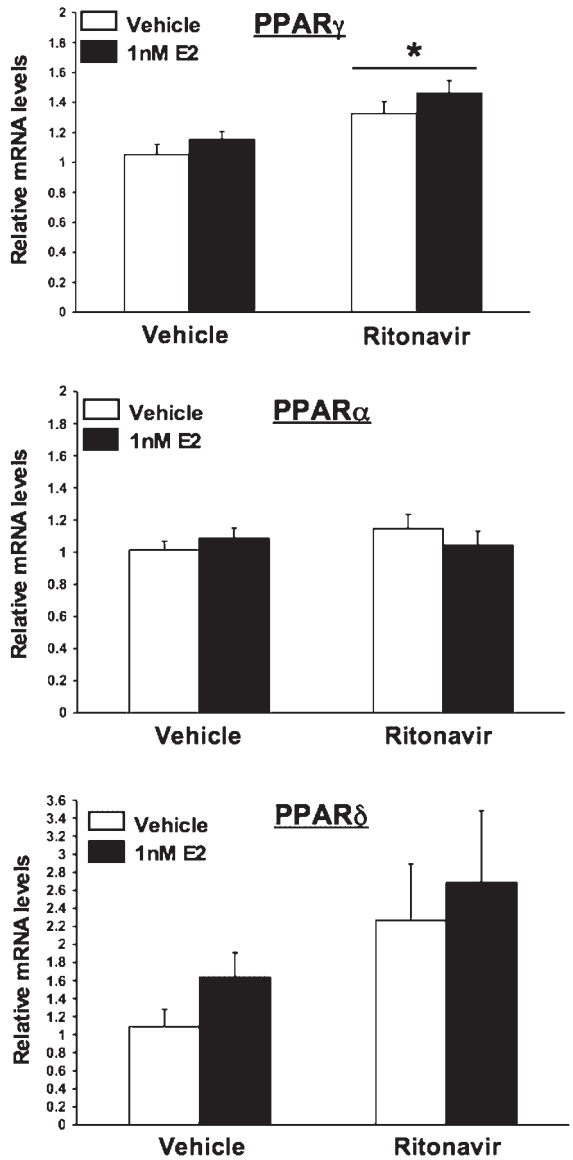

Fig. 3. Ritonavir increases PPARγ mRNA expression. Differentiated THP-1 cells were pretreated with vehicle (0.01% EtOH) or 1 nM E2 for 24 h and then incubated with 50 μg/ml of agLDL along with 30 ng/ml ritonavir or vehicle (0.01% ethanol) for 24 h. Cells were lysed, RNA isolated and real-time RT-PCR performed. All data was normalized to an internal housekeeping control gene and expressed relative to the vehicle/vehicle control. Bars represent mean ± SEM, n = 3. Asterisk (*) significantly different from vehicle ($P < 0.05$).

CD36 protein expression by immunocytochemistry (Fig. 4A). Cells were treated as described above, however, they were seeded on glass cover slips and fixed for 10 min in 4% paraformaldehyde at the conclusion of the treatment. Fluorescent intensity of staining was quantified from 10 random fields per cover slip and normalized to the number of cells in the field as determined by DAPI staining of nuclei (Panel b). Negative controls with primary and secondary antibodies omitted were also included (Panel c and d). Ritonavir increased CD36 protein expression correlating with the mRNA expression ($P < 0.05$; Fig. 4B). Cells pretreated with E2 had significantly less CD36 protein expression ($P < 0.05$). To confirm the immunocytochemistry data, we also performed western immunoblot analysis of CD36 from THP-1 cells treated with ritonavir in the presence or absence of E2 (Fig. 4C). Western immunoblots showed a similar pattern of CD36 protein levels as observed by immunocytochemistry.

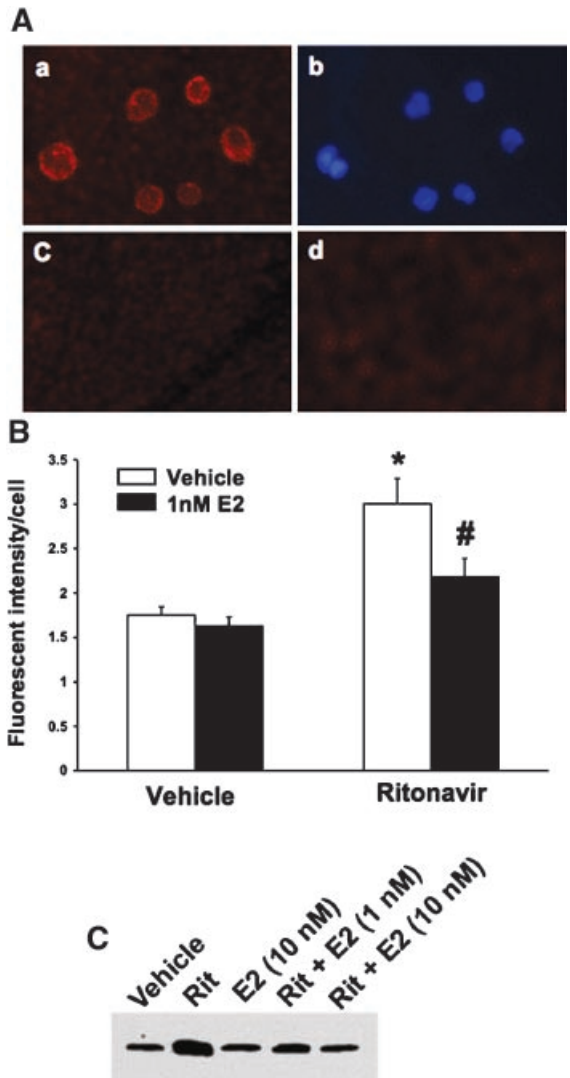

Fig. 4. E2 prevents the ritonavir-induced increase in CD36 protein expression. Immunocytochemistry was used to identify CD36 protein expression on THP-1 cells treated as described in Figure 2. Representative micrographs are shown (**A Panel a**, CD36 expression; **b**, DAPI; **c**, minus primary antibody; **d**, minus secondary antibody). Magnification = 200×. The relative fluorescent intensity of immunocytochemical staining was measured using ImagePro software (**B**). Bars represent the relative intensity per cell ± SEM, n = 4–6. Asterisk (*) significantly different from the vehicle/vehicle group ($P < 0.05$). # significantly different from vehicle/ritonavir ($P < 0.05$). To confirm overall changes in CD36 expression western immunoblot analysis was also performed using the same primary antibody (**C**). A representative micrograph is shown.

DISCUSSION

In the present study we have extended the understanding of the molecular mechanisms involved in the ability of estrogen to influence cholesterol metabolism in macrophages. Using the human monocyte/macrophage cell line, THP-1, we determined that estrogen can directly prevent the accumulation of cholesteryl esters induced by the HIV protease inhibitor, ritonavir. Furthermore, estrogen prevents the increase in CD36 expression at the level of protein expression.

Cholesterol ester accumulation in macrophages is a critical step in the formation of foam cells, fatty streaks, and ultimately atherosclerosis. Preventing the accumulation of cholesteryl esters is one of the many mechanisms by which estrogen contributes to the prevention of atherosclerosis. Estrogen has also been shown to increase nitric oxide release, resulting in vasodilation and a decrease in blood pressure and to modify lipoprotein production in the liver [Mendelsohn and Karas, 1999; Dubey et al., 2002; Seed and Knopp, 2004]. Estrogen has previously been shown to regulate cholesterol uptake in macrophages [Sulistiyani and St Clair, 1997; McCrohon et al., 1999; Seed and Knopp, 2004], but this is the first demonstration that it also does so in the face of a drug that induces cholesteryl accumulation. Previous studies have shown that long-term treatment with estrogen decreases scavenger receptor affinity for acetylated LDL in human-derived macrophages [Sulistiyani and St Clair, 1997; McCrohon et al., 1999]. In the current study, we did not observe changes in basal cholesteryl ester accumulation with estrogen treatment in the

absence of ritonavir. This may be due to the length of treatment with estrogen [Napolitano et al., 2002]. In the present study the treatment with estrogen is relatively short-term.

Despite recent data from the Women's Health Initiative that indicates that current hormone replacement strategies can cause cardiovascular complications when given to older postmenopausal women [Rossouw et al., 2002], other clinical studies have shown that estrogen has cardioprotective actions [Grodstein et al., 1996; Mendelsohn and Karas, 1999, 2005]. Clinical data suggests that both estrogen and progesterone contribute to the gender protection in terms of cardiovascular disease, although there is some evidence that progesterone can antagonize the atheroprotective actions of estrogen [Writing Group for the PEPI Trial, 1995]. To examine these interactions at the level of the macrophage, we pretreated cells with either hormone alone or in combination and examined the effect of ritonavir. Progesterone had no effect by itself and also failed to reverse the effect of estrogen. This would indicate that any antagonistic effects of progesterone on the overall development of atherosclerosis in response to ritonavir, do not occur at the level of the macrophage. Other studies have suggested that progesterone prevents cholesteryl ester synthesis in human monocyte-derived macrophages and inhibits cellular translocation of free cholesterol in J774 cells [Mazzone et al., 1995; McCrohon et al., 1999]. A small, but insignificant decrease in cholesteryl esters was observed with progesterone treatment alone. It is possible that progesterone does play as similar role in THP-1 cells, but other mechanisms may mask any progesterone action. The assay employed in these studies did not address synthesis or movement of cholesteryl esters.

The scavenger receptor CD36 is an essential component in the formation of ritonavir-induced cholesterol laden macrophages, foam cell formation and ultimately atherosclerosis [Dressman et al., 2003]. Ritonavir increases CD36 mRNA by increasing PPARγ activation in macrophages. Others have shown, however, that ritonavir decreases CD36 expression in undifferentiated THP-1 cells [Serghides et al., 2002]. One possible explanation for this difference is that during the differentiation process, factors involved in regulating CD36 expression are altered. This is a potentially interesting difference that may provide insights into differential effects of ritonavir in monocytes and macrophages. Previously we demonstrated that ritonavir has less of an effect on CD36 mRNA levels in macrophages from female mice [Allred et al., 2006]. In the THP-1 cells however, estrogen did not alter CD36 mRNA levels with or without ritonavir treatment. This difference is likely due to a possible combination of additional hormonal and cytokine factors in female mice.

Members of the PPAR family of transcription factors have been shown to be intimately involved in the regulation of various aspects of metabolism and cholesterol homeostasis (for review see Moore et al. [2001]). It has previously been shown that ritonavir increases PPARγ expression and this increase is responsible for the increase in CD36 [Dressman et al., 2003]. In the present study, estrogen had no effect on this mechanism. Furthermore, ritonavir selectively increases PPARγ and not other members of the family, suggesting a specific effect of ritonavir on PPARγ signaling. Interestingly, PPARγ did not stimulate LXRα mRNA levels and in turn ABCA1, suggesting that the effects of ritonavir are multi-faceted and not due to a global increase in all PPARγ functions. Estrogen treatment alone did not regulate PPARs, RXRα, or LXRα expression. Other studies have shown that in macrophages the withdrawal of estrogen causes an increase in LXRα mRNA levels [Kramer and Wray, 2002]. The timing and duration of estrogen treatment may explain such differences observed in gene expression in our studies.

Our data indicate that in the presence of ritonavir, CD36 protein expression can be independently regulated by estrogen. CD36 has been shown to be regulated at the level of translation in a hyperglycemic model of diabetes [Griffin et al., 2001]. Elevated glucose levels increase the translational efficiency of CD36 mRNA in macrophages. Estrogen has also been shown to modulate genes at the level of mRNA translation [Wu et al., 2003]. In the adrenal gland, estrogen decreases angiotensin AT1 receptors that play a role in the prevention of hypertension. Whether this is a mechanism by which estrogen can regulate CD36 levels remains to be seen. Since estrogen alone had no effect, the actions of estrogen are only unmasked with the increase in transcription and/or translation stimulated by ritonavir. Alternatively, high levels of ritonavir are

known to inhibit proteasome activity that can result in the over-expression of CD36 [Munteanu et al., 2005] suggesting that CD36 levels can be modified at the protein level. Estrogen can increase proteasome activity in cells of macrophage lineage [Reed et al., 2004]. Thus it is also possible that estrogen could reverse any potential effects of ritonavir on the proteasome or have direct actions on proteasome activity in THP-1 cells. This is currently being investigated. Given the proven role of CD36 in cholesterol metabolism following ritonavir treatment, this is likely the mechanism of estrogen action. It remains possible, however, that E2 has additional effects not tested in the present study.

The data presented in this study describes, for the first time, the ability of estrogen to alter cholesterol metabolism in response to ritonavir. In particular estrogen can prevent the increase in CD36 protein expression induced by the HIV protease inhibitor, ritonavir. This study adds to the multitude of data on the varied actions of estrogen in the cardiovascular system and in particular, in the development of atherosclerosis.

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