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Stimulation of CD36 and the key effector of reverse cholesterol transport ATP-binding cassette A1 in monocytoid cells by niacin

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Abstract

Niacin, the first lipid lowering drug shown to improve survival after myocardial infarction, decreases LDL and increases HDL cholesterol levels. These effects cannot fully be explained by its suspected mechanism of action, inhibition of lipolysis and hepatic VLDL synthesis. Niacin has also been shown to interfere with the cyclic AMP (cAMP)/protein kinase A (PKA) pathway and massively stimulate prostaglandin D_2 (PGD_2) formation. The major metabolite of PGD_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), was recently identified as the most potent endogenous PPARγ activator. We, therefore, studied the effects of niacin on the PPARγ- and cAMP-dependent expression of receptors promoting reverse cholesterol transport. The transcription of PPARγ-, HDL-, LDL- and scavenger-receptors and the sterol exporter ABCA1, were measured by quantitative RT-PCR and cellular cholesterol efflux and PPARγ activation studied in macrophage and hepatocyte models. Niacin stimulated the translocation of PPARγ and the transcription of PPARγ, CD36 and ABCA1 in monocytoid cells, whereas the LDL-receptor (LDL-R) was unchanged. Thereby niacin enhanced HDL-mediated cholesterol efflux from the cells resulting in a reduced cellular cholesterol content. The niacin effect on CD36 but not on ABCA1 was prevented by cyclooxygenase inhibition, whereas the niacin effect on ABCA1 but not on CD36 was prevented by PKA inhibition, suggesting mediation by the 15d-PGJ_2/PPARγ and the cAMP/PKA pathways, respectively. These new actions of niacin on several key effectors of reverse cholesterol transport out of the vessel wall provide a rational to expect regression of atherosclerosis and test the combination of niacin with statins for an overadditive clinical benefit.

Keywords: Nicotinic acid; Scavenger receptors; ABCA1; Reverse cholesterol transport; HDL; PPARγ

1. Introduction

Nicotinic acid (niacin), as vitamin B3 an essential micronutrient, has long been used in pharmacological doses as a hypolipidemic agent. It has been shown to improve long-term survival after myocardial infarction in the Coronary Drug Project [1]. This sustained benefit suggests stabilisation or even regression of the atherosclerotic process by niacin. Niacin lowers plasma levels of triglycerides, total and LDL cholesterol and increases HDL levels. Inhibition of peripheral lipolysis and hepatic VLDL synthesis, shunt apolipoprotein B (apo B) degradation and decreased apo A-I removal have been demonstrated as mechanisms of action [2–4]. A reduction of cyclic AMP (cAMP) has been suspected to underly the inhibition of lipolysis in adipocytes [5].

The dose limiting side effect of niacin, vasodilation with flushing, is mediated by a severalfold increase of systemic PGD_2 levels [6]. PGD_2 is the major prostaglandin in monocytes and monocytes are a major source of PGD_2 formation. By autocrine and paracrine binding to its plasma membrane receptor PGD_2 can stimulate cellular cAMP levels [7]. In addition, the main cellular metabolite of PGD_2, 15d-PGJ_2 [8], is regarded as the most potent endogenous ligand of PPARγ, a transcription factor for several genes involved in lipid metabolism [9]. Among them are CD36, the quantitatively most important scavenger receptor

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Abbreviations: ABCA1, ATP-binding cassette A1; apo A-I, apolipoprotein A-I; BSA, bovine serum albumin; cAMP, cyclic adenosine monophosphate; dHDL, delipidated high density lipoprotein; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; HDL, high density lipoprotein; LDL, low density lipoprotein; LDL-R, low density lipoprotein receptor; Lox-1, lectin-like oxidised low-density lipoprotein receptor-1; oxLDL, oxidised low density lipoprotein; PGD_2, prostaglandin D_2; PKA, protein kinase A; PPARγ, peroxisome proliferator activated receptor gamma; RT-PCR, reverse transcription-polymerase chain reaction; SR-B1, scavenger receptor-B1; VLDL, very low density lipoprotein.

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for uptake of oxidised lipoproteins into macrophages [10], the ABCA1 protein [11,12], the key transporter for the efflux of cellular cholesterol to apolipoprotein A-I containing particles, and SR-B1 serving also as HDL-receptor [13,14]. The expression of ABCA1 in macrophages is enhanced not only by the transcription factor PPARγ [15] but also by cAMP [16]. We therefore studied, whether niacin directly or by stimulating PGD_2 and subsequent signalling pathways enhances the expression of CD36 and ABCA1 and reverse cholesterol transport out of macrophages. Such an additional peripheral mechanism of action of niacin in the vessel wall would complement the effects of statins and provide a biochemical rational for the synergistic clinical benefit from the combination of both drugs.

2. Materials and methods

2.1. Cell culture

The human differentiated monocytic cell line Mono Mac 6 (MM6sr) [17] was used as a macrophage model and maintained in RPMI 1640 medium containing 10% foetal calf serum, insulin (9 μg/mL), oxaloacetate (1 mmol/L), pyruvate (1 mmol/L), penicillin (200 U/mL), streptomycin (200 μg/mL), nonessential amino acids (1×), and L-glutamine (2 mmol/L). For every experiment, the cells were seeded at a density of 0.2×10^6 cells/mL in 24-well plastic culture dishes (Falcon) with 2 mL of medium. The human hepatoblastoma cell line HepG2, a standard model of hepatic lipid metabolism [4], was grown in flasks (Nunc) in Dulbecco's modified Eagles medium containing 10% foetal calf serum, 25 mM glucose, 110 mg/L L-glutamine and sodium pyruvate and passaged every third day. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of healthy donors by Ficoll (Biochrom AG) gradient centrifugation and seeded in 2 mL RPMI 1640 medium at a density of $5–10 \times 10^6$ cells. Cells were incubated for 3 or 48 hr in control medium only or in the presence of nicotinic acid (1000 μM, Sigma). For inhibitor experiments, cells were preincubated for 30 min in the presence of 100 μM acetylsalicylic acid (ASA; Sigma) or the PKA inhibitor Ro 31-8220 (900 nM, Upstate Biotechnology) [18], before niacin or the cAMP analogue cBIMPS (100 μM, BIOLOG Life Science Institute) [19] was added. No toxic effects on cell viability were detected by microscopy and by the annexin V receptor binding assay for apoptosis measured by FACS after incubation of cells with niacin concentrations up to 5000 μM for at least 48 hr.

2.2. RNA isolation and amplification with RT-PCR

Total cellular RNA was isolated from 2×10^6 MM6sr cells, 2×10^6 HepG2 cells or $5–10 \times 10^6$ PBMCs with the Quantum Prep AquaPure RNA Isolation Kit (BioRad) and treated with DNAse (Promega). Specific primers were synthesised by Metabion GmbH as previously described for β-actin, CD36 [20], LDL-receptor (LDL-R) [21], Lox-1 [22] and ABCA1 [15]. For SR-B1 and PPARγ newly designed primers were used (SR-B1 forward: 5′-CTTCCTCGAGTACCGCAC-3′, reverse: 5′-GAGCCACGAAGCGATAGG-3′; PPARγ forward: 5′-CATGGCAATTGAATGTCGTG-3′, reverse: 5′-CCTCAAATATGGAGTCCACG-3′). Comparison of the selected primers (BLAST search) [23] with the human GenBank sequences revealed high specificity. Ready-To Go RT-PCR Beads (Amersham Pharmacia) were used for reverse transcription and PCR amplification. The amplificates were separated by ethidium bromide-stained (Roth) agarose gel electrophoresis for qualitative control. Specific amplificates were quantified by HPLC separation on a DEAE ion-exchange column (Perkin-Elmer) with a solvent gradient of 300–600 mM NaCl buffered at pH 9 and UV detection by absorption at 260 nm [22] using the β-actin amplificates for normalisation (Figs. 2 and 3).

For later experiments a real-time cycler became available (iCycler, BioRad) and specific amplificates were detected by fluorescence (SybrGreen I, Molecular Probes), quantified using β-actin as internal standard [24] and plotted as percent of control experiments (Figs. 4, 6 and 7B).

2.3. Cell fractionation and Western blotting

Cytosolic and nuclear cell fractions from MM6sr cells were always prepared from the same cell sample as described [25] and protein content was measured by the D_C Protein Assay (BioRad). Western blots were performed with a primary antibody for PPARγ (New England Biolabs), goat anti-rabbit IgG-HRP (Santa Cruz) and a chemiluminescence detection system (Pierce).

2.4. Flow cytometry

MM6sr cells were incubated with 1000 μM niacin or control medium for 48 hr, washed with Hank's Balanced Salt Solution (Sigma) containing 10 mM Hepes, 1 mM Ca^{2+}, 1 mM Mg^{2+} and 0.5% BSA (Sigma). For PPARγ staining, MM6sr cells were permeabilised with ice-cold methanol for 1 min. After blocking unspecific binding with 5% human serum for 30 min, MM6sr cells were incubated with an irrelevant mouse IgG (MOPC 21, Sigma) or anti-PPARγ (clone E-8, Santa Cruz) for 45 min on ice, reacted with secondary FITC-conjugated anti-mouse IgG for 30 min (Sigma), fixed with 3.7% formaldehyde and analysed by flow cytometry.

2.5. Lipoprotein preparation and cholesterol efflux assay

Lipoproteins were prepared and oxidised as described [26]. HDL was delipidated (dHDL) with methanol and

diethylether [27]. MM6sr cells were preincubated for 24 hr with 1000 μM niacin or control medium and then 50 μg/mL of desalted oxidised LDL (oxLDL) or control medium was added for lipid exposure of cells for 48 hr.

For efflux experiments MM6sr cells were preincubated for 24 hr with 1000 μM niacin or control medium and then for 24 hr 20 μg/mL dHDL was added. After incubation MM6sr cells were washed twice with PBS and the cellular pellet was extracted with chloroform/methanol and cholesterol was measured enzymatically (Cholesterin liquicolor, Rolf Greiner).

2.6. Cyclic AMP measurement

1×10^7 MM6sr cells were washed three times with PBS to remove all RPMI 1640 medium and were incubated for 30 min with 1000 μM niacin, Cicaprost (100 nM, Schering AG, Berlin) as cAMP stimulant or carrier control. Cyclic AMP was extracted and measured according to the manufacturer's protocol (direct cAMP, EIA kit, Assay Designs).

2.7. Statistical analysis

Independent experiments with concurrent controls were analysed by Students *t*-test.

3. Results

For the qualitative assessment of the effects of niacin on PPARγ and the receptors studied, MM6sr cells were incubated for 48 hr with niacin or control medium. Then RNA was extracted and amplified by RT-PCR. A gel separation of the amplificates stained with ethidium bromide revealed an enhanced transcription of PPARγ and CD36 message after niacin incubation compared to control, whereas β-actin and LDL-R transcription was unchanged (Fig. 1A). In parallel, cytosolic and nuclear protein fractions were prepared from cells and Western blots for PPARγ were made. In control cells, more PPARγ was detected in the cytosol compared to the nucleus, in cells incubated with niacin more PPARγ was detected in the nucleus compared to the cytosol (Fig. 1B). Staining of permeabilised cells with a PPARγ antibody demonstrated a moderately increased specific total fluorescence of cells after niacin incubation (Fig. 1C).

For studying the time course and quantifying the niacin effects on the transcription of PPARγ and the receptors of interest, MM6sr cells were incubated with niacin for 3 and 48 hr or in control medium. RNA was extracted immediately and the specific transcripts were measured by quantitative RT-PCR. Niacin significantly increased PPARγ transcription about 2-fold after 3 hr and about 3.5-fold after 48 hr (Fig. 2A). Simultaneously, niacin significantly increased transcription of CD36 (Fig. 2B) and ABCA1 (Fig. 2C) about 2-fold at 3 and 48 hr.

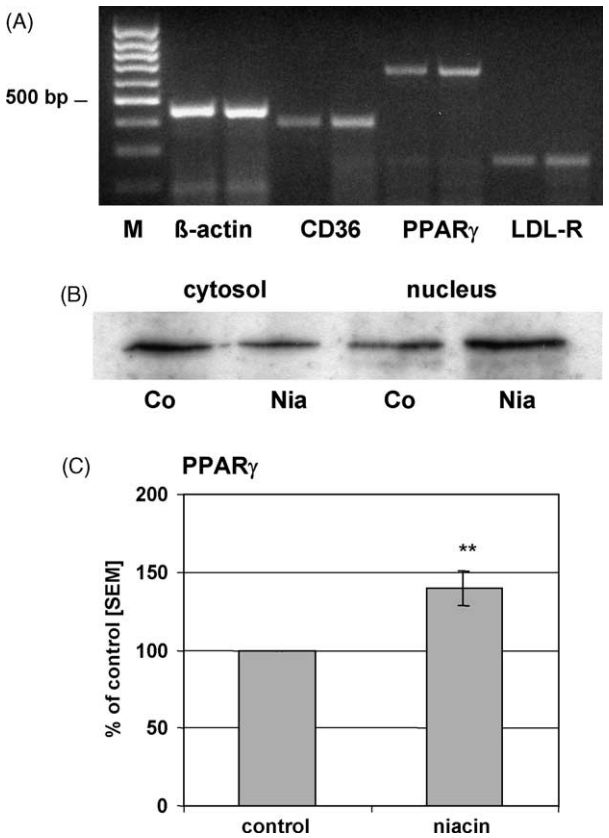

Fig. 1. Qualitative effects of niacin on β-actin, CD36, PPARγ and LDL-R transcription, PPARγ protein expression and localisation in MM6sr cells after 48 hr: (A) ethidium bromide stained agarose gel separation of specific RT-PCR amplificates of β-actin, CD36, PPARγ, and LDL-R mRNA from MM6sr cells kept under control conditions (left lanes of each pair) or from cells incubated with niacin (right lanes); (B) Western blot of PPARγ in protein extracts prepared from the cytosolic (lanes 1 and 2) and nuclear compartment (lanes 3 and 4) of MM6sr cells kept under control conditions (lanes 1 and 3) and of cells incubated with niacin (lanes 2 and 4); (C) specific mean fluorescence as measured by FACS scan in permeabilised MM6sr cells stained for PPARγ after incubation under control conditions (left) or in the presence of niacin (right) (means ± SEM, N = 7; $^{**}P < 0.01$).

Similarly, niacin significantly stimulated ABCA1 transcription in hepatic HepG2 cells about 2.5-fold after 3 hr and 1.5-fold after 48 hr (Fig. 3A), whereas SR-B1 transcription was not changed by niacin (Fig. 3B). Except for a marginal about 10% of LDL-R increase in MM6sr cells after 48 hr niacin incubation, niacin had also no effects on LDL-R and Lox-1 transcription in monocytoid or hepatic cells (data not shown).

To support the potential clinical relevance of the stimulation of CD36 and ABCA1 transcription, we also studied the effects of niacin on CD36 and ABCA1 in normal human monocytes. Peripheral monocytes were prepared from eight healthy donors and incubated with niacin or carrier. In monocytes from all eight individuals niacin uniformly stimulated CD36 and ABCA1 transcription. The effect was already pronounced after 3 hr of niacin exposure (Fig. 4).

Next, the functional effect of the niacin-induced changes of receptors and transporters involved in cellular cholesterol

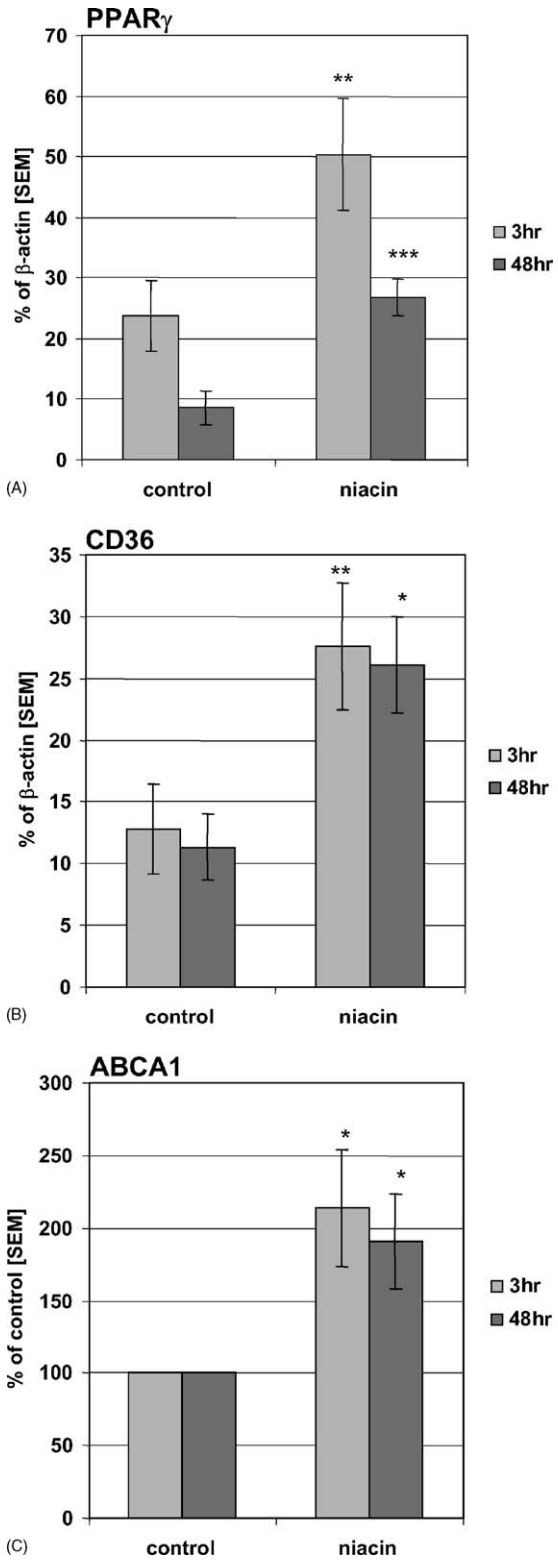

Fig. 2. Quantification and time course of effects of niacin on PPARγ, CD36 and ABCA1 transcription in MM6sr cells: β-actin normalised levels of mRNA specific for PPARγ (A), CD36 (B) and ABCA1 (C) measured by quantitative RT-PCR in MM6sr cells kept under control conditions (left pairs of columns) or after incubation with niacin (right pairs of columns). Levels after 3 hr are indicated by grey columns and levels after 48 hr by dark columns (means ± SEM, $N \geq 6$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

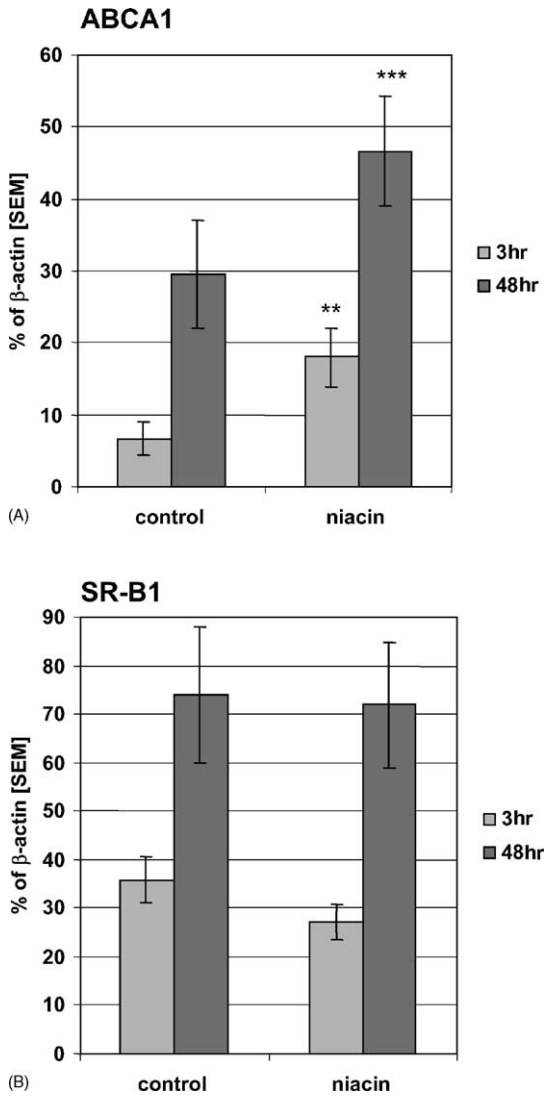

Fig. 3. Quantification and time course of effects of niacin on ABCA1 and SR-B1 transcription in hepatic cells: β-actin normalised levels of mRNA specific for ABCA1 (A) and SR-B1 (B) measured by quantitative RT-PCR in HepG2 cells kept under control conditions (left pairs of columns) or after incubation with niacin (right pairs of columns). Levels after 3 hr are indicated by grey columns and levels after 48 hr by dark columns (means ± SEM, $N = 7$; $^{**}P < 0.01$, $^{***}P < 0.001$).

handling were studied. MM6sr cells were preincubated either with niacin or with control medium alone for 24 hr, then oxidised LDL was added for 48 hr, cells were washed twice, extracted and the cellular cholesterol content was determined. Niacin significantly reduced the cholesterol content of cells kept in standard medium as well as in medium enriched with oxidised LDL (Fig. 5A). Similarly, MM6sr cells were preincubated either with niacin or with control medium alone for 24 hr and then delipidated HDL (dHDL) was added for 24 hr as an acceptor for cellular cholesterol. Niacin significantly enhanced the cellular cholesterol efflux to delipidated HDL particles above the efflux to dHDL from cells in niacin-free medium (Fig. 5B).

To explore the mechanisms of CD36 and ABCA1 stimulation by niacin MM6sr cells were preincubated

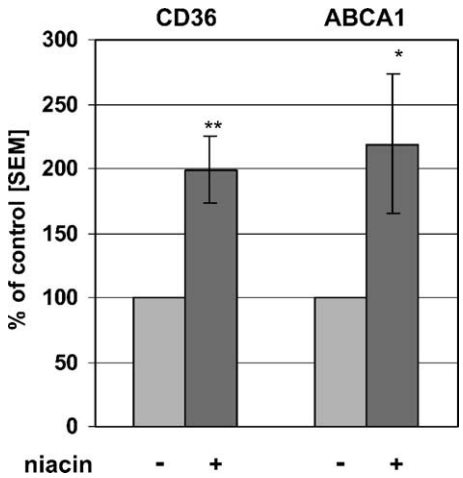

Fig. 4. Effects of niacin on CD36 and ABCA1 transcription in human monocytes: levels of mRNA specific for CD36 (left) and ABCA1 (right) measured by quantitative RT-PCR in freshly prepared peripheral monocytes kept under control conditions (grey columns) or after incubation with niacin (dark columns) after 3 hr (means ± SEM, N = 8; $^{*}P < 0.05$, $^{**}P < 0.01$).

for 30 min with specific inhibitors or agonists of cell signalling pathways suspected to be involved before niacin or control medium was added for 3 hr. Preincubation of cells with the cyclooxygenase inhibitor ASA reduced baseline CD36 transcription in MM6sr cells and completely prevented the stimulation of CD36 by niacin (Fig. 6A). ASA also reduced baseline ABCA1 transcription but did not reduce the stimulation of ABCA1 by niacin (Fig. 6B). This supported an obligatory role of a cyclooxygenase product, presumably PGD_2 and its metabolite 15d-PGJ_2, in the niacin stimulation of CD36, but not of ABCA1.

Therefore, cells were also preincubated with Ro 31-8220, a PKA inhibitor. Ro 31-8220 had no effect on basal and niacin stimulated CD36 transcription (Fig. 6A). In contrast, Ro 31-8220 reduced baseline ABCA1 transcription and completely prevented the stimulation of ABCA1 by niacin (Fig. 6B). In further control experiments, cBIMPS, a cAMP mimic, stimulated ABCA1 similar to niacin and this could also completely be prevented by Ro 31-8220 (Fig. 6C).

As this suggested a positive role of the cAMP/PKA pathway in the niacin stimulation of ABCA1 in monocytes/macrophages, we directly measured cAMP levels in MM6sr cells. After incubation of cells with niacin for 30 min cAMP levels were significantly increased by about 30% compared to control cells not exposed to niacin (Fig. 7A). Furthermore, LXRα was tested as a further PPARγ regulated gene and found to be increased on niacin incubation of cells by over 40% after 3 hr (Fig. 7B).

4. Discussion

The mechanisms underlying the increase of plasma HDL cholesterol, that might be most important for the

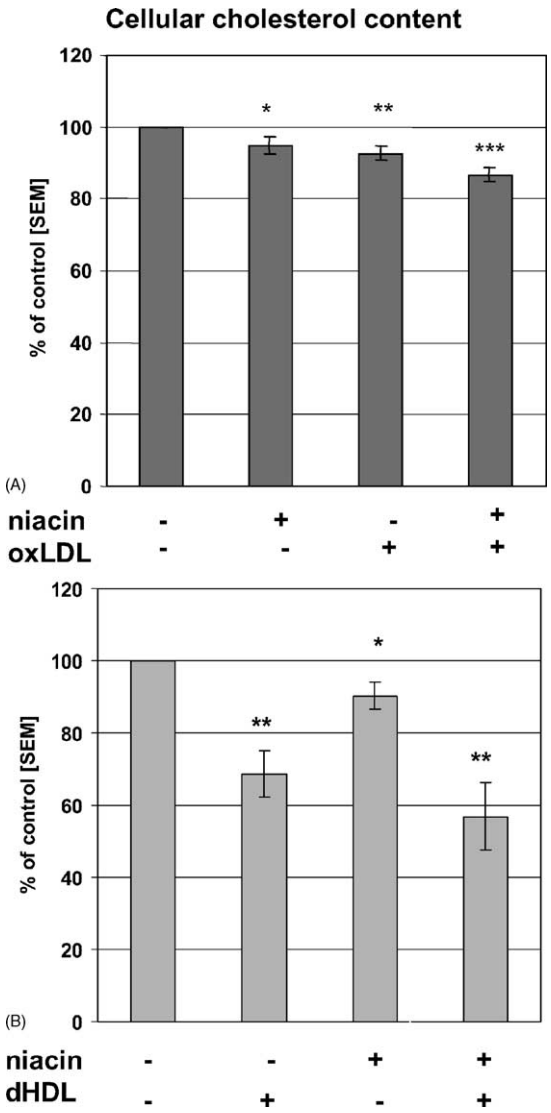

Fig. 5. Effects of niacin on cellular cholesterol content and HDL-stimulated cholesterol efflux from MM6sr cells. (A) From left to right: cholesterol content of cells kept in control medium, in the presence of niacin, oxidised LDL or the combination of both. (B) From left to right: cellular cholesterol at baseline, after exposure to delipidated HDL, after exposure to niacin or after exposure to the combination of both (means ± SEM, N = 4; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

clinical benefit from niacin, have only rarely been addressed. As niacin is known to massively stimulate PGD_2 synthesis [6], PGD_2 increases cellular cAMP levels and the major PGD_2 metabolite, 15d-PGJ_2, is the most potent endogenous PPARγ activator identified so far [9], we had hypothesised, that niacin should stimulate PPARγ- and cAMP-dependent key effectors of reverse cholesterol transport in monocytoid cells.

Indeed, niacin was found to stimulate transcription of PPARγ, nuclear translocation and total cellular content of PPARγ protein in human monocytoid cells. Consistently with this, niacin was also found to increase CD36 and ABCA1 transcription in monocytoid cells and ABCA1 in hepatic cells, whereas LDL-R and Lox-1 transcription, but

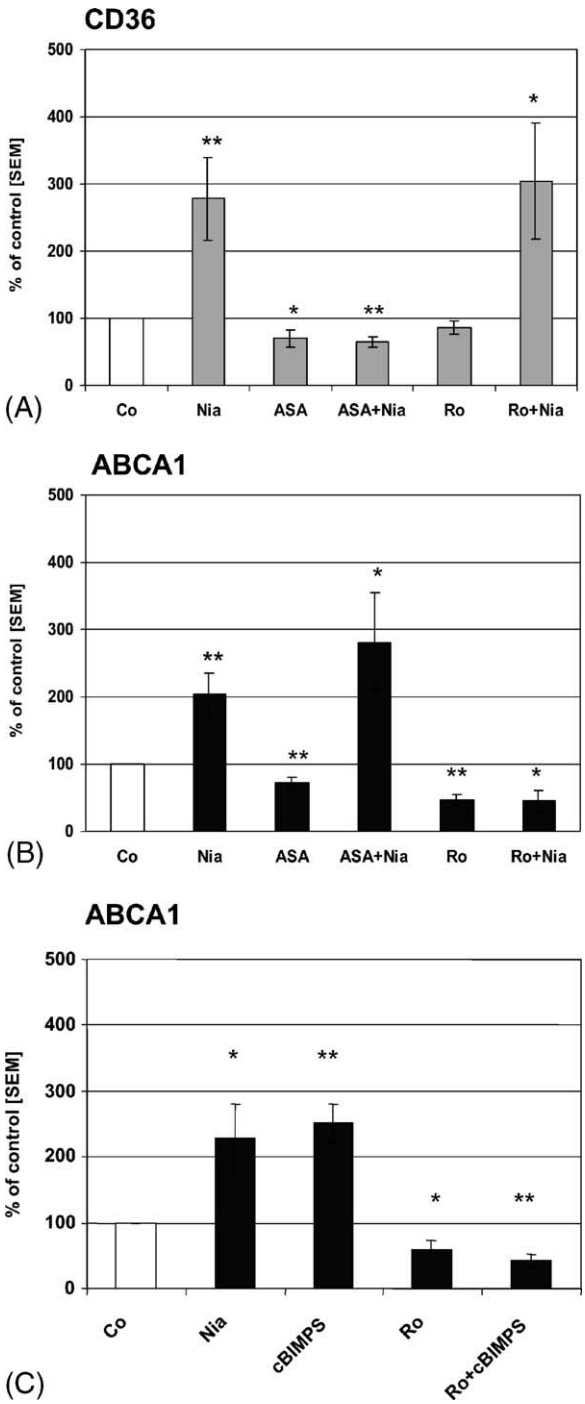

Fig. 6. Effects of inhibitors and agonists of the prostaglandin/PPARγ and cAMP/PKA pathways on niacin effects in MM6sr cells. Transcription of CD36 (A) and ABCA1 (B) under control conditions, after incubation with niacin, ASA, ASA combined with niacin, the PKA inhibitor Ro 31-8220 and the combination with niacin. (C) Transcription of ABCA1 under control conditions, after incubation with niacin, the cAMP mimic cBIMPS, the PKA inhibitor Ro 31-8220 and the combination of it with cBIMPS, adequate concurrent carrier controls were use for each blocker and agonist (means ± SEM, $N \geq 6$; $^{*}P < 0.05$, $^{**}P < 0.01$).

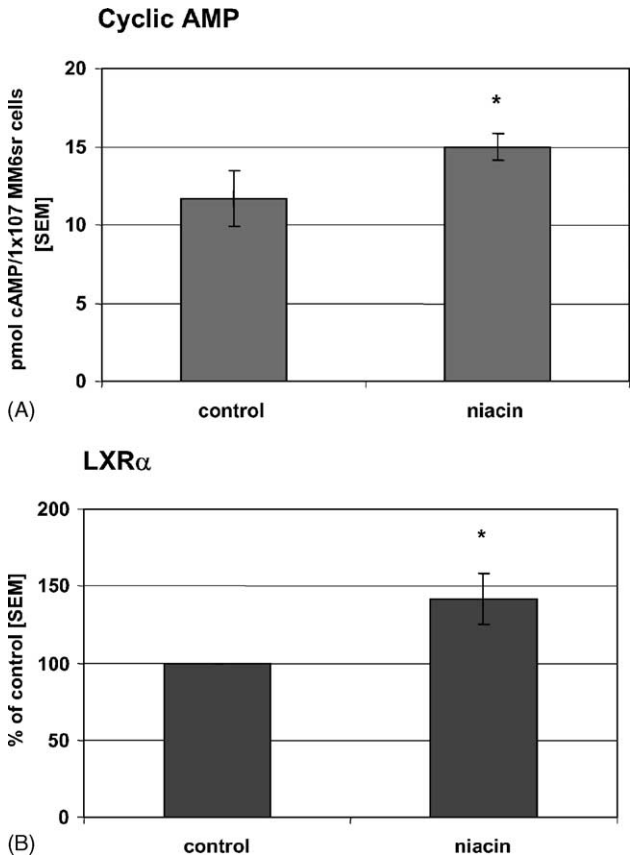

Fig. 7. Effects of niacin on cyclic AMP levels and on LXRα transcription in MM6sr cells. (A) Monocytoid cells were incubated for 30 min with niacin (right column) or control medium (left column) and extracted for cyclic AMP (means ± SEM, $N = 5$; $^{*}P < 0.05$). (B) MM6sr cells were incubated for 3 hr with niacin (right column) or control medium (left column) and β-actin normalised LXRα mRNA was measured by RT-PCR (means ± SEM, $N = 7$; $^{*}P < 0.05$).

also SR-B1 in hepatic cells, was not affected by niacin. The niacin effect was evident as early as 3 hr after exposure and sustained at least for 48 hr and could be confirmed in freshly prepared human peripheral monocytes. In fact, CD36 [10], ABCA1 [15] and SR-B1 [14] have been shown to be at least partially controlled by PPARγ activation, whereas LDL-R transcription is feed-back regulated by the intracellular cholesterol concentration sensed via sterol responsive element binding proteins [28]. The regulation of Lox-1 differs from that of other scavenger receptors in several ways [22].

Induction of CD36 by niacin will enhance the capacity of macrophages to take up modified lipoproteins and therefore stimulate the removal of modified lipids accumulated in the extracellular compartment of the vessel wall [29]. The PPARγ agonist rosiglitazone-induced CD36 expression which was however not proatherogenic in an animal model [30]. The intracellular uptake of cholesterol and niacin will then simultaneously enhance ABCA1 expression. ABCA1 has been shown to transport cellular cholesterol to the outer leaflet of the plasma membrane, from where it is easily transferred to apo A-I containing HDL particles. Enhanced ABCA1 transcription thus counteracts foam cell formation by initiating reverse cholesterol transport [31]. The functional relevance of the niacin-induced changes in CD36 and ABCA1 expression could

indeed be demonstrated. Niacin significantly reduced cellular cholesterol content, enhanced the cells ability to withstand overloading with modified LDL particles and enhanced HDL-mediated cholesterol efflux. The pathophysiologic importance of ABCA1 activity for cellular cholesterol export has also been demonstrated in other models [31] and a correlation of ABCA1 activity to intima media thickness an early sign of atherosclerosis, has already been established [32].

To complete reverse cholesterol transport, HDL particles have to be deloaded by the liver, the only organ capable of metabolising and excreting cholesterol. In human HepG2 cells, an accepted model of hepatic lipid metabolism, niacin induced ABCA1 transcription but had no significant effect on SR-B1. The stripping of lipids from HDL particles by hepatic lipase was long thought to constitute the decisive mechanism. Later SR-B1, initially described as a scavenger receptor homologue to CD36 on monocytes/macrophages, was characterised as a hepatic HDL receptor abstracting lipids from HDL particles but not internalising apo A-I [13]. The unchanged expression of SR-B1 is compatible with the maintained hepatic cholesterol uptake and reduced apo A-I removal observed in HepG2 cells after niacin [3]. ABCA1 is highly expressed in the liver [33], but its role in hepatic cholesterol metabolism is incompletely understood. ABCA1 may be involved in hepatic cholesterol excretion into the bile as ABCA1 transgenic mice had increased cholesterol concentrations in the bile [34]. In ABCA1 knock out mice, however, hepatobiliar cholesterol transport appeared not to be impaired [35]. In rodents, niacin might increase hepatic bile acid production indirectly by enhancing expression of the 7-α-cholesterol hydroxylase via PPARγ-induced expression of LXRα [36]. This should, however not be relevant in man as the human 7-α-cholesterol hydroxylase promotor lacks a LXRα responsive element.

The experiments with specific inhibitors and agonists to elucidate the signalling pathways that transmit the niacin effects supported the role of niacin-induced PGD_2 formation and PPARγ activation for CD36 expression. In contrast, niacin-induced ABCA1 expression was not inhibited by cyclooxygenase blockade with ASA. This renders a mediation by a cyclooxygenase product like PGD_2 unlikely. As cAMP has been shown to induce ABCA1 expression [16], we tested the effect of a PKA inhibitor on niacin-induced ABCA1 stimulation. PKA inhibition prevented the niacin-induced ABCA1 stimulation and niacin increased cellular cAMP levels in our macrophage model. In adipocytes lowering of lipolysis by niacin has been shown to be mediated by activation of a G_i protein inhibiting adenylate cyclase. A putative niacin receptor has been characterised in mouse macrophages. A specific guanine nucleotide sensitive binding site for [^{3}H]nicotinic acid was detected in membranes from mouse RAW 264.7 macrophages [37]. In mouse adipocytes this niacin binding site was recently identified as the orphan G protein-coupled receptor (PUMA-G) and shown to reduce isoproterenol stimulated cAMP levels in a pertussis toxin inhibitable manner [38]. The effects of niacin on basal cellular cAMP levels without isoproterenol stimulation were not reported, but in adipocytes niacin was shown to inhibit lipolysis by a G_i protein-mediated inhibition of adenylate cyclase [39]. In man, two nicotinic acid receptors have recently been identified by a G_i-protein guided strategy but these receptors were however not expressed in macrophages [40] and therefore cannot mediate the effects of niacin in our cells. The moderate increase of cAMP levels by niacin observed in our macrophage model does not necessarily imply activation of a small receptor-coupled G_s protein, but rather other signal transduction mechanism may indirectly increase cAMP. The promotor structure of ABCA1 suggests a complex regulation [41] and indirect effects of niacin on one of these pathways may induce ABCA1. For example, PPARγ has been shown to induce expression of LXRα protein, an oxysterol activated transcription factor, that then in turn can induce ABCA1 transcription [42].

The present study revealed effects of niacin on the transcription of several key transporters and receptors involved in reverse cholesterol transport. These effects were shown to be functionally relevant leading to a reduced cellular cholesterol content and to enhanced HDL-mediated cholesterol efflux from monocytoid cells. This new peripheral site of action of niacin may contribute to the marked increase of HDL cholesterol by niacin and complements the actions of statins. Thus, it deserves testing whether by this new effect niacin could provide a synergistic clinical benefit in the combination with statins, that could be overadditive to the combined effects on lipoprotein levels [43,44].

Acknowledgments

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