FEBS Letters 583 (2009) 1274–1280

FEBS Letters

journal homepage: www.FEBSLetters.org

Micellar lipid composition profoundly affects LXR-dependent cholesterol transport across CaCo2 cells

Michele Petruzzelli [a,b,c,d,e], Albert K. Groen [a,b], Karel J. van Erpecum [a,c], Carlos Vrins [a,b], Astrid E. van der Velde [a], Piero Portincasa [d], Giuseppe Palasciano [d], Gerard P. van Berge Henegouwen [c], Giuseppe Lo Sasso [e], Annalisa Morgano [e], Antonio Moschetta [d,e,*]

[a] Department of Experimental Hepatology, Academic Medical Center, Amsterdam, The Netherlands
[b] Department of Medical Biochemistry, Academic Medical Center, Amsterdam, The Netherlands
[c] Department of Gastroenterology and Hepatology, University Hospital Utrecht, The Netherlands
[d] Clinica Medica "A. Murri", Department of Internal and Public Medicine, University of Bari, Italy
[e] Laboratory of Lipid Metabolism and Cancer, Consorzio Mario Negri Sud, Via Nazionale 8/A, 66030 Santa Maria Imbaro (Chieti), Italy

ARTICLE INFO

Article history:
Received 18 December 2008
Revised 12 February 2009
Accepted 11 March 2009
Available online 20 March 2009

Edited by Laszlo Nagy

Keywords:
ABC-A1
Liver X receptor
Bile salt
Intestine
Lyso-phosphatidylcholine
Phosphatidylcholine

ABSTRACT

Intraluminal phospholipids affect micellar solubilization and absorption of cholesterol. We here study cholesterol transport from taurocholate–phospholipid–cholesterol micelles to CaCo2 cells, and associated effects on ABC-A1 mediated cholesterol efflux. Micellar incorporation of egg-yolk-phosphatidylcholine markedly increased apical retention of the sterol with decreased expression of ABC-A1, an effect that is prevented by synthetic liver X receptor (LXR) or retinoid X receptor (RXR) agonists. On the other hand, incorporation of lyso-phosphatidylcholine (LysoPC) increased ABC-A1–HDL-dependent basolateral cholesterol efflux, an effect that is abated when LXR is silenced. Thus, the modulation of cholesterol metabolism via intraluminal phospholipids is related to the activity of the oxysterol nuclear receptor LXR.

1. Introduction

In humans on a Western diet, daily dietary cholesterol intake is 300–500 mg. Approximately twice this amount of cholesterol reaches the duodenum through biliary secretion [1]. Intestinal cholesterol absorption increases serum cholesterol levels, as well as hepatic uptake and biliary secretion of the sterol. Cardiovascular disease, non-alcoholic steatohepatitis and cholesterol gallstones are potential consequences of excessive intestinal cholesterol absorption. Whereas most dietary fats are almost completely absorbed, humans absorb only 30–65% of ingested cholesterol. Recent research has greatly enhanced insight in processes involved in regulating intestinal cholesterol uptake: Niemann-Pick C1-like-1 (NPC1L1) appears to function as the major protein facilitating uptake of cholesterol [2] and the half-transporters ABC-G5 and ABC-G8 mediate partial secretion of cholesterol within the enterocyte back to the intestinal lumen, thus decreasing net absorption [3]. At the basolateral side, intracellular cholesterol is transferred in esterified form to the core of chylomicrons. In addition, recent studies in the apolipoprotein-A1−/− mouse model [4] and in mice specifically and selectively lacking ABC-A1 in the intestine [5] have yielded compelling evidence that significant amounts of unesterified cholesterol may be delivered through an apolipoprotein-A1 dependent pathway to the circulation [6]: ABC-A1 is located at the basolateral membrane of the enterocyte and other polarized cell types [7–10] and may deliver unesterified cholesterol to apolipoprotein-A1 containing particles in the circulation. ABC-G1 is another member of the ABC-transporter family and its role in reverse cholesterol transport has been mainly investigated in macrophages [11,12]. In the intestine, ABC-G1 is expressed at the basolateral

Abbreviations: ABC, ATP-binding casette; DMEM, Dulbecco's minimum essential medium; FCS, fetal calf serum; HDL, high density lipoprotein; LysoPC, Lyso-phosphatidylcholine; LXR, liver X receptor; NPC1L1, Niemann-Pick C1-like-1; PC, Phosphatidylcholine; RXR, retinoid X receptor; SM, sphingomyelin; TC, taurocholate

* Corresponding author. Address: Laboratory of Lipid Metabolism and Cancer, Consorzio Mario Negri Sud, Via Nazionale 8/A, 66030 Santa Maria Imbaro (Chieti), Italy. Fax: +39 0872 570299.
E-mail addresses: moschetta@negrisud.it, a.moschetta@dimimp.uniba.it (A. Moschetta).

0014-5793/$36.00
doi:10.1016/j.febslet.2009.03.021

membrane of the enterocyte, its expression levels are highest in cells of the upper villi, and it is induced by excess dietary cholesterol [13]. Although at present the exact role of ABC-G1 in the intestine is unknown, it has been shown in vitro that its function is similar to ABC-A1; ABC-G1 mainly interacts with mature circulating high density lipoprotein (HDL) particles, to which it delivers cholesterol [14].

The transcription factor liver X receptor (LXR) senses cholesterol overload and restricts its accumulation by upregulating the transcription of genes involved in cellular elimination (ABC-A1 and ABC-G1), hepatic delivery (lipoprotein lipase, cholesteryl ester transfer protein), and intestinal or biliary excretion (ABC-G5 and ABC-G8) of cholesterol [15]. Although less well appreciated, intestinal cholesterol solubilization in mixed bile salt–phospholipid micelles also influences cholesterol absorption profoundly. Micellar cholesterol solubility largely depends on phospholipid contents, phospholipid acyl chain composition and phospholipid class [16,17]. Effects of phospholipid contents are illustrated in the *mdr-2* "knockout" mouse with absent biliary phospholipids, due to deficient *mdr-2* protein that normally functions as a floppase for phospholipid over the hepatocytic canalicular membrane [18]. These mice display markedly decreased intestinal cholesterol absorption [19]. The principal phospholipid in the gastrointestinal tract is bile-derived phosphatidylcholine (PC), which exceeds diet-derived PC by as much as 5:1 [20]. Most of this PC is digested by pancreatic phospholipase-A2, and the resulting LysoPC is efficiently absorbed. Of note, conversion into LysoPC enhances cholesterol absorption markedly [21–23]. PC in human bile contains mainly 16:0 acyl chains at the *sn*-1 position and mainly unsaturated (18:2 > 18:1 > 20:4) acyl chains at the *sn*-2 position [24]. In presence of bile salts, PC with saturated acyl chains (e.g. dipalmitoyl phosphatidylcholine) exhibits much lower cholesterol micellization capacity (with preferential formation of cholesterol-phospholipid vesicles) [17]. Also, phospholipase-A2 induced hydrolysis is much lower under these circumstances [25], with decreased intestinal cholesterol absorption as a result [20,26]. Similarly, sphingomyelin (SM) – a major phospholipid in the outer membrane hemileaflet present in considerable quantities in Western diets – has a strong affinity for cholesterol and is incompletely digested in the gastrointestinal tract [27]. Also, sphingomyelin has been shown to decrease intestinal cholesterol absorption [20,26]. Recently, Field and colleagues showed that in intestinal cells, the influx of apical membrane cholesterol enhances gene and protein expression of ABC-A1, resulting in increased efflux of cellular cholesterol to apoAI and HDL production [28]. We hypothesize that phospholipids in the intestinal lumen may profoundly affect micellar cholesterol solubilization thereby affecting cholesterol uptake in the enterocyte and availability of ligands for the nuclear receptor LXR, with significant effects on intestinal cholesterol trafficking. We study in the current work effects of micelles of various compositions on cholesterol absorption and expression of various relevant genes in CaCo2 cells.

2. Materials and methods

2.1. Preparation of lipid solutions

Lipid mixtures containing variable proportions of bile salts, phospholipids and cholesterol (stock solutions in methanol for bile salts, in chloroform for phospholipids and cholesterol) were vortex-mixed and dried at 45 °C under a mild stream of nitrogen, and subsequently lyophilized during 24 h, before being dissolved in aqueous solutions. Tubes were sealed with Teflon-lined screw caps under a blanket of nitrogen to prevent lipid oxidation and vortex-mixed for 5 min followed by incubation at 37 °C in the dark. All solutions were warmed up to 45 °C for 10 min and subjected to sonication (three pulses during 2 s, at 40 Hz) before use. The final mol percentages of cholesterol, phospholipid and bile salts did not differ more than 1% from the intended mol percentages.

2.2. Lipid analysis

Phospholipid concentrations in model systems were assayed by determining inorganic phosphate according to Rouser. Cholesterol concentrations were determined with an enzymatic assay, and bile salts with the 3α-hydroxysteroid dehydrogenase method [29]. In some experiments, phospholipids were extracted [30], separated by thin-layer chromatography (chloroform–methanol–acetic acid–water 50:25:8:2, vol/vol/vol/vol), and quantified by determination of phosphorus contents.

2.3. Cholesterol efflux experiments

CaCo2 cells were grown into polycarbonate micropore membranes (3.0 μm pore size, 24 mm diameter) inserted into transwells (Costar, Cambridge MA, USA). Two weeks after confluency, cells were incubated overnight in presence of model biles (taurocholate (TC) 5 mM, phosphatidylcholine or lyso-phosphatidylcholine (LysoPC) 0.2 mM and chol 0.1 mM according to previous publications) [21] plus 0.5 μCi/well of [^{3}H] cholesterol. Model biles were added to the upper chamber, while the lower chamber received fetal calf serum (FCS)-free Dulbecco's minimum essential medium (DMEM)/PBS in presence or absence of 35 μg/mL HDL or 20 μg/mL of Apolipoprotein-A1 (ApoA1). After overnight incubation, an aliquot of upper chamber media was collected, while all lower chamber media was collected after insert removal. Media from both chambers were centrifuged at 14000 rpm for 5 min. Remaining upper chamber media was discarded and unincorporated labelled cholesterol was removed by extensive washing twice with PBS containing 0.2% bovine serum albumin, and once with HBSS. Thereafter, polycarbonate membranes were cut from the inserts and placed in scintillation vials. The quantity of [^{3}H] cholesterol in upper media, lower media and polycarbonate membranes was determined by liquid scintillation counting.

2.4. Adenoviral constructs

Short hairpin human LXRα adenovirus (shLXRα) was generated based on a validated sequence (available upon request) from a set of four Upgrade duplex Dharmacon using the BLOCK-iT U6 RNA entry vector kit (Invitrogen). ShLXRα oligos were synthesized with complementary ends to be subcloned into the pENTR/U6 shuttle vector. Then, the pENTR/U6 shLXRα plasmid was recombined with the destination vector pAd/BLOCK-iT-DEST to generate an adenovector containing shLXRα under the control of the U6 promoter. The oligos to generate the shLacZ vector were purchased from Invitrogen. ShLXRα and shLacZ adenovectors were linearised with Pac I restriction enzyme and the digested products, after purification with Qiaex II gel extraction kit (Qiagen Inc., Chatsworth, CA), were used to transfect 293A cells (Invitrogen) with Lipofectamine 2000 (Invitrogen) to generate the corresponding adenoviruses. The viruses were propagated into 293A cells. Adenoviral titer was determined by RTqPCR with specific primers [31]. Crude viral lysate stocks were stored at −80 °C until use. ShLXRα adenovirus was validated both on LXRα mRNA and protein levels as well as on ABC-A1 mRNA levels after incubation with a synthethic LXR ligand (Fig. S1A–C).

2.5. Statistical analysis

Values are expressed as means ± SEM of 3–6 separate experiments. Differences between groups were tested for statistical significance by Mann Witney-*U* tests or analysis of variance

(ANOVA) with the aid of NCSS software (Kaysville, UT) as appropriate. When ANOVA detected a significant difference, results were further compared for contrasts using Fisher's least significant difference test as post-hoc test. Statistical significance was defined as two-tailed probability of less than 0.05.

Details on the chemicals used and methods for cell culture incubation, Western blot analysis, real time quantitative PCR with primer list are available as supplementary on line materials.

3. Results

3.1. Cholesterol absorption experiments

According to absorption experiments in transwell systems, incorporation of PC or SM into apical TC–cholesterol micelles strongly increased retention of the radioactive cholesterol tracer in the apical compartment (90% of total: Fig. 1A). Incorporation of LysoPC led to less apical retention compared to PC or SM (62% vs 90% of total: Fig. 1A), with reciprocal changes in the cellular compartment (Fig. 1B). Of note, incorporation of LysoPC into apical micelles also increased basolateral H^3-cholesterol efflux in presence of HDL, but not in absence of this acceptor (Fig. 1C). Micellar SM or PC incorporation did not change basolateral cholesterol efflux. Also in presence of ApoA1 as basolateral acceptor, LysoPC containing micelles increased basolateral cholesterol efflux, while no effect was evident for micelles containing SM or PC (Fig. S2A). In a time-course experiment, we showed that induction of basolateral cholesterol efflux by LysoPC containing micelles is maximal after 24 h (Fig. S2B).

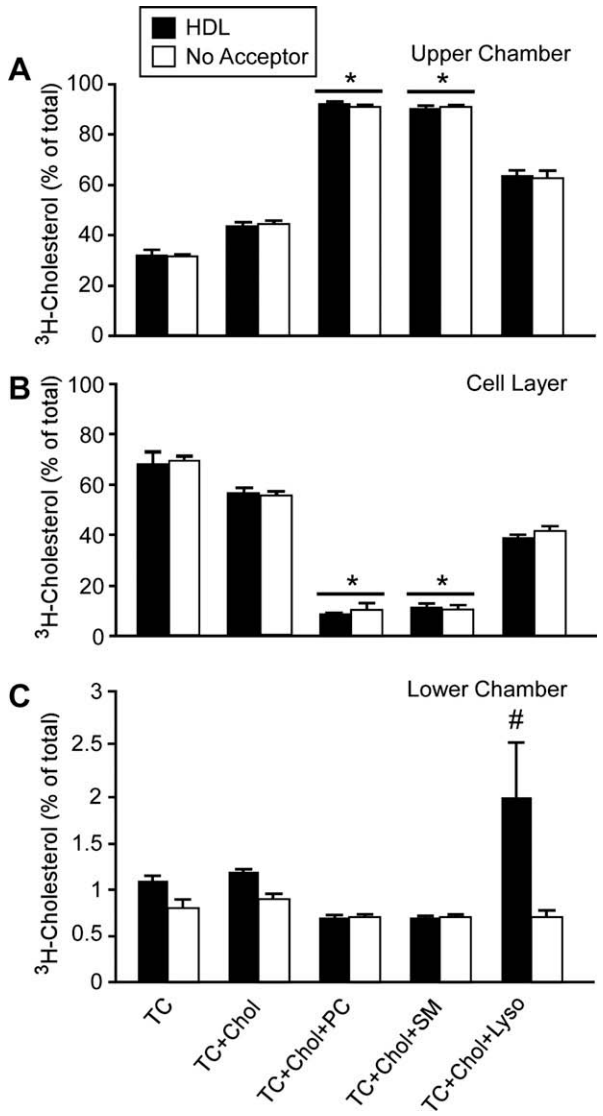

Fig. 1. Effects of micellar phospholipids on apical retention (A), cellular uptake (B) and basolateral efflux (C) of ^{3}H-cholesterol. Sphingomyelin or phosphatidylcholine inhibit cellular uptake of the tracer, which is less the case for lyso-phosphatidylcholine. Lyso-phosphatidylcholine enhances basolateral efflux of H^3 cholesterol only in presence of HDL as basolateral acceptor. *Significantly different from TC, TC + cholesterol and TC + cholesterol + LysoPC micelles in both HDL and no HDL conditions; #Significantly different from no HDL condition. Black bar with HDL and white bar without HDL added to the basolateral compartment.

3.2. Effects of incubating CaCo2 cells with various micelles on ABC transporters

As shown in Fig. 2A, incubation of CaCo2 cells with TC simple micelles (5 mM final conc.) did not alter ABC-A1 mRNA expression, while incubation with PC alone (1 mM final conc.) resulted in slight but significant decrease. In contrast, incubation with TC–PC micelles (5 mM and 1 mM final bile salt and phospholipid conc. resp.) induced a fivefold decrease of ABC-A1 mRNA. Incorporation of SM in the bile salt micelles at the same concentration tended to reduce ABC-A1 mRNA even further. When PC content in TC micelles (5 mM final conc.) was varied (PC 0.5, 1, 2, 3, 4 mM final conc.), progressive and dose-dependent decreases of ABC-A1 mRNA levels were observed (results not shown). Thin layer chromatography of supernatant after overnight incubation of CaCo2 cells with TC–PC or TC–SM micelles (final bile salt conc. 5 mM, final phospholipid conc. 1 mM) revealed that only minimal amounts (approximately 2%) of EYPC had been converted into LysoPC, while SM remained entirely unmodified (data not shown). Incorporation of cholesterol (1 mM final conc.) in the micelles exerted opposite effects compared to phospholipid incorporation, with now twofold increased ABC-A1 mRNA expression (Fig. 2B). Additional incorporation of PC or (even more pronounced) SM (1 mM final conc.) into the TC–cholesterol micelles decreased ABC-A1 mRNA expression again. When we incubated CaCo2 cells with TC–LysoPC–cholesterol micelles (TC 5 mM + LysoPC 1 mM + Cholesterol 1 mM), ABC-A1 mRNA expression increased markedly to about seven times control values. Western blots after incubation with various model biles largely mimicked mRNA data (Fig. 2C).

As shown in Fig. 2D, incubation with TC simple micelles (5 mM final conc.) induced a slight and with PC a more pronounced decrease of ABC-G1 mRNA expression. Incorporation of PC or (more pronounced) SM (at 1 mM final conc.) in the TC micelles further decreased ABC-G1 mRNA expression. When PC content in TC micelles (5 mM final conc.) was varied (PC 0.5, 1, 2, 3, 4 mM final conc.), decreases of ABC-G1 mRNA levels did not depend on amount of the phospholipid (results not shown). Additional incorporation of cholesterol did not lead to marked changes of ABC-G1 mRNA expression compared to corresponding conditions without cholesterol (Figs. 2E versus 2D). With LysoPC within TC–cholesterol micelles (TC 5 mM + LysoPC 1 mM + cholesterol 1 mM), there was no change in ABC-G1 expression compared to control condition, while a significant increase was observed when compared to PC- or SM-containing micelles (Fig. 2E).

We next sought to evaluate whether the increased expression of ABC-A1 after TC + Chol + LysoPC was determined at transcriptional levels, and if the transcriptional response was direct or it implied the synthesis of additional transcription factors or proteins. To this aim, we incubated CaCo2 cells with model biles in presence of the gene transcription inhibitor actinomycin D (final conc. 5 μg/mL) and protein synthesis inhibitor cycloheximide (final conc. 500 μM). In presence of actinomycin D, no difference in ABC-A1 mRNA levels were observed between control incubation (i.e. no

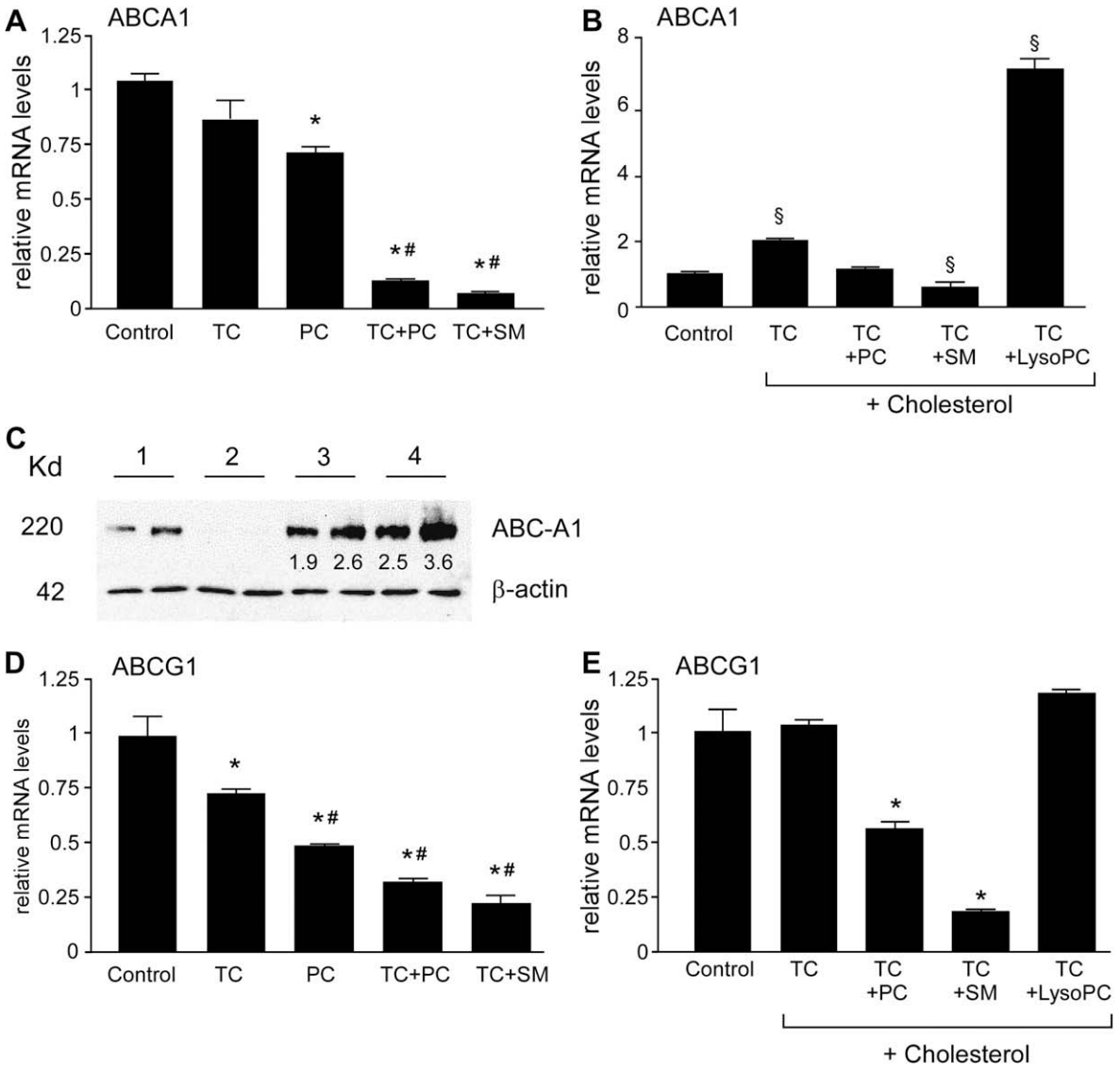

Fig. 2. ABC-A1 mRNA expression after overnight incubation of Caco2 cells with various model biles without (A) or with (B) cholesterol. Significantly different from *control and #TC and PC; §significantly different from all other conditions. (C) ABC-A1 protein level as determined by Western blot after overnight incubation of Caco2 cells with various model biles with or without cholesterol. A representative experiment is shown ($N = 3$). Condition 1: control; Condition 2: TC+PC; Condition 3: TC+Chol; Condition 4: TC+Chol+LysoPC. ABC-G1 mRNA expression after overnight incubation of Caco2 cells with various model biles without (D) or with (E) cholesterol. *Significantly different from control; #significantly different from TC.

lipid micelles) versus TC + Chol simple micelles, nor micelles composed of PC or LysoPC (Fig. 3A). On the contrary, in presence of cycloheximide, TC + Chol simple micelles doubled ABC-A1 expression compared to control incubation; also, the addition of PC to TC + Chol micelles inhibited ABC-A1 expression, while the addition of LysoPC increased ABC-A1 transcripts threefold (Fig. 3A).

Preincubation during 6 h with the synthetic LXR agonist T0901317 (final conc. 1 μM) induced approximately 30-fold increase of ABC-A1 mRNA expression (Fig. 3B). Subsequent overnight incubation with TC–PC micelles (TC 5 mM + PC 1 mM conc.) in presence of the LXR agonist revealed unchanged expression of ABC-A1 transporter. Interestingly, even in presence of LXR agonist, incubation with TC–Chol–LysoPC micelles (TC 5 mM + LysoPC 1 mM + cholesterol 1 mM) was still able to induce a twofold increase in ABC-A1 mRNA levels, thus underlining a putative additive effect of LysoPC incubation and synthetic agonists. Preincubation during 6 h with the natural RXR ligand 9-*cis* retinoic acid (final conc. 1 μM) induced approximately eightfold increase of ABC-A1 (Fig. 3B) mRNA levels. While subsequent overnight incubation with TC–PC model biles (5 mM and 1 mM final conc. resp.) in presence of the RXR ligand slightly reduced ABC-A1 expression, incubation with TC–Chol–LysoPC micelles (TC 5 mM + LysoPC 1 mM + cholesterol 1 mM) resulted in no difference compared to DMSO.

Lastly, to prove that the LXR transcriptional pathway is directly involved in the effects of micellar phospholipids on ABC-A1 expression, we incubated cells with model biles in presence of shLXRα. LXRa knockdown decreased by 50% the ABC-A1 induction by TC–Chol–LysoPC micelles (TC 5 mM + LysoPC 1 mM + cholesterol 1 mM) compared to control incubation with AdshLacZ (Fig. 4A). We also evaluated the effect of incubation with lipid micelles in presence of AdshLXRα or AdshLacZ on LXRα and LXRβ gene expression levels. TC–Chol–LysoPC micelles doubled LXRα expression levels, the effect being completely abolished in presence of AdshLXRα (Fig. 4B). Also, LXRα expression levels after TC–Chol were halved when cells were infected with of AdshLXRα compared to AdshLacZ. In contrast to LXRα, expression levels of LXRβ were unmodified neither by incubation with different micelles, nor infection with AdshLXRα (Fig. 4C) and might account for the residual ABC-A1 induction via TC–Chol–LysoPC micellar incubation.

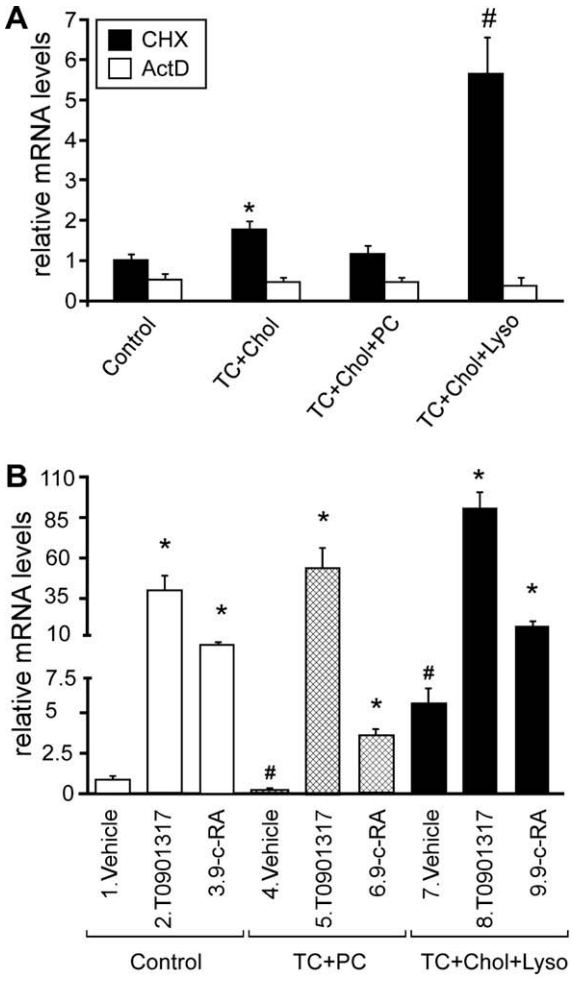

Fig. 3. (A) ABC-A1 mRNA expression after overnight incubation of CaCo2 cells with various model biles (TC 5 mM, PC/LysoPC 1 mM, cholesterol 1 mM) in presence of gene transcription inhibitor actinomycin D (final conc. 5 μg/mL), and after 12 h co-incubation with protein synthesis inhibitor cycloheximide (final conc. 500 μM); black bars: cycloheximide; white bars: actinomycin D. (B) ABC-A1 mRNA expression after overnight incubation of CaCo2 cells with various model biles with 6 h preincubation with synthetic LXR agonist T0901317, or natural retinoid X receptor ligand 9-cis retinoic acid (final conc. 1 μM). *Significantly different from vehicle (bars 1, 4 and 7); #significantly different from vehicle (bar 1).

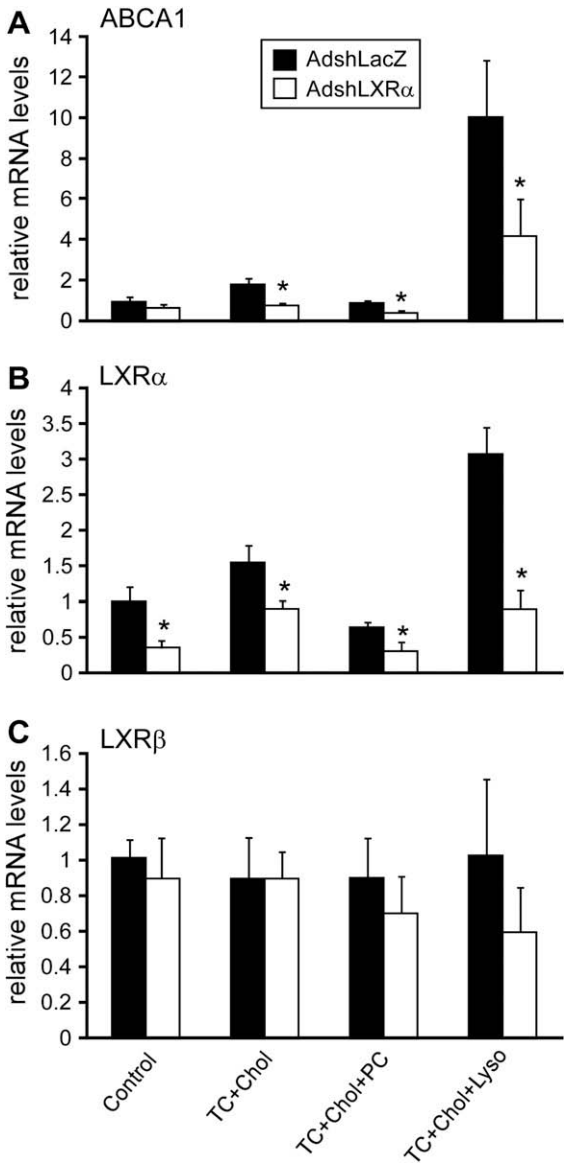

Fig. 4. ABC-A1 (A), LXRα (B), and LXRβ (C) mRNA expression after overnight incubation of CaCo2 cells with various model biles and after cell infection with AdshLacZ or AdshLXRα. *Significantly different from shLacZ.

We also evaluated potential effects of incubation with TC micelles (5 mM final conc.), with or without phospholipid (PC or LysoPC, 1 mM final conc.) with or without cholesterol (1 mM final conc.) on CaCo2 cell mRNA expression of SRB1, NPC1L1P, ABC-G5 and ABC-G8. There were no significant changes of SRB1 or NPC1L1 mRNA (results not shown). When considering ABC-G5 or ABC-G8, only very low mRNA levels were found in CaCo2 cells, with or without various micellar incubations and whether or not LXR agonist had been added, as previously reported by others [14] (results not shown).

4. Discussion

Class, content and acyl chain composition of phospholipids in the intestinal lumen may affect cholesterol absorption, with important implications for dietary prevention of hypercholesterolemia. In presence of bile salts, PC with unsaturated acyl chains exhibits high, while saturated phospholipids (e.g. dipalmitoyl PC, SM) low cholesterol micellizing capacity [17]. Of note, conversion of PC into LysoPC enhances cholesterol absorption markedly [22,23]: by its more hydrophilic nature, LysoPC is contained less tightly in bile salt micelles, which results in less tight packing of sterols in the micelles, thus allowing enhanced transfer of cholesterol from the micellar phase through the unstirred water layer to the enterocyte [21]. In our current experiments with CaCo2 cells, we found only minimal LysoPC formation after overnight incubation with PC–bile salt micelles, indicating minimal or absent phospholipase-2 activity. Therefore, we performed experiments not only with PC- and SM-containing bile salt micelles, but also with LysoPC–bile salt micelles. To prevent cytotoxicity, cholesterol was incorporated within these micelles as well [32,33]. In line with various in vivo and in vitro data [21–23], we found that incorporation of PC or SM into TC–cholesterol micelles that were added to the apical side of CaCo2 cells markedly increased apical retention of radioactive tracer cholesterol, whereas incorporation of LysoPC enhanced cellular uptake of the tracer cholesterol.

In complementary experiments, we found that overnight incubation with TC–PC micelles significantly and dose-dependently decreased ABC-A1 expression in CaCo2 cells both at mRNA and protein level. In contrast, incorporation of cholesterol or LysoPC + cholesterol strongly enhanced ABC-A1 expression.

The different effects of various micellar phospholipids upon cholesterol trafficking and ABC-A1 gene expression are likely to be mediated by modulating availability of cholesterol for apical uptake by the enterocyte and subsequent modifications of the intracellular oxysterol pool. Also, in intestinal cells, the influx of apical membrane cholesterol enhances gene and protein expression of ABC-A1, resulting in increased efflux of cellular cholesterol to apoAI and HDL production [28]. Since CaCo2 cells are able to synthesize and secrete apolipoprotein-A1 [34], one may speculate that cellular cholesterol may efflux to apolipoprotein-A1 containing PC–TC or SM–TC micelles in the apical compartment. When LysoPC is present in lipid micelles, we found enhanced HDL/ApoA1-dependent cholesterol efflux at the basolateral side. These findings point to the relevance of the recently discovered HDL-mediated pathway of intestinal cholesterol uptake [4–6]. In our study we observe an increase in intracellular cholesterol levels when cells are incubated with both TC–cholesterol–LysoPC and with TC–cholesterol micelles. Nevertheless, while TC–cholesterol–LysoPC micelles increase LXR activity, ABC-A1 expression and HDL-ApoA1 basolateral cholesterol efflux, TC–cholesterol micelles do not activate LXR. We think that apart from the enhanced uptake of the sterol, the effect of LysoPC containing micelles are likely due to increased activation of LXR by oxysterols rather than increased substrate (free cholesterol) availability. Measurements of intracellular concentration of oxysterols are needed in the future to prove this hypothesis.

Oxysterols are the natural ligands of LXR. This nuclear receptor is highly expressed in the intestine and is thought to act as a sterol sensor: in case of excess cellular cholesterol influx, it activates genes that transcribe proteins eliminating or limiting accumulation of cellular cholesterol [35]. Recognized gene targets of LXR include various ABC membrane transporters such as ABC-A1, ABC-G1, ABC-G5 and ABC-G8 [3,35]. In line with previous data [14], we found in the current study high levels of both LXRα and LXRβ, while very low mRNA levels for ABC-G5 or ABC-G8 were found in CaCo2 cells with or without various micellar incubations, with or without LXR–RXR stimulation. We found in the CaCo2 cell model that preincubation with pharmacologic amounts of LXR agonist or RXR ligand markedly increased ABC-A1 and ABC-G1 expression, in line with previous studies [14]. Effects of micellar lipids were blunted in presence of actinomycin D, a general inhibitor of gene transcription, thus proving that regulation of ABC-A1 by micellar lipids involves transcriptional events. To determine whether the modulation of ABC-A1 expression by micellar phospholipids represents a direct transcriptional effect or whether it involves the synthesis of different transcription factors or proteins, we evaluated the effects of micellar phospholipids in presence of cycloheximide. Even in presence of this protein synthesis inhibitor, micellar PC inhibited, while LysoPC enhanced ABC-A1 expression. This finding would suggest that the modulation of ABC-A1 expression by micellar phospholipids represents a direct transcriptional effect.

To prove that such direct transcriptional effect is sustained by LXR activation, we employed both gain and loss of function approaches. Gain of function experiments with the synthetic LXR agonist T0901713 showed that LXR activation prevents micellar-PC-induced inhibition of ABC-A1 expression. Interestingly, the increase of ABC-A1 expression upon LXR stimulation was even further enhanced by micellar LysoPC. Since the transcriptional activity of LXR is dependent upon its dimerization with the retinoid X receptor (RXR), we evaluated the effects of micellar lipids in presence of the RXR natural ligand 9-*cis* retinoic acid. Similarly to LXR activation, 9-*cis* retinoic acid stimulation prevented micellar-PC-induced ABC-A1 down-regulation, while no effects were evident for micellar LysoPC enhancement of ABC-A1 gene expression. The recovery by LXR and RXR agonists of the inhibitory effect of micellar-PC on ABC-A1 expression demonstrates the involvement of these nuclear receptors in the process. Lastly, the role of LXR for LysoPC-dependent induction of ABC-A1 expression was documented employing a loss of function approach. Silencing LXR activity by the employment of an adenovirus strongly diminishes ABC-A1 gene induction by micellar LysoPC, thus demonstrating the importance of LXR in the process.

In conclusion, the present study indicates that micellar phospholipid content and class markedly affect cholesterol absorption, HDL-dependent efflux and the expression of involved genes in the CaCo2 cell model. Employing gain and loss of function approaches, plus experiments with transcription and protein synthesis inhibitors, we suggest a model where the effects of intraluminal micellar phospholipids on cholesterol metabolism in CaCo2 cells are directly related to the presence and activation of oxysterol nuclear receptor LXRα. These findings may be relevant for dietary modification of intestinal cholesterol absorption.

Acknowledgements

We thank Drs. A. Kosters, W. Renooj, M. de Smet, N.G. Venneman for their help during the study. This study was supported in part by a "Meelmeyergrant" from the Academic Medical Center in Amsterdam, by Grants 912-02-063 from the Netherlands Organization for Scientific Research and MW 04-55 form the Netherlands Digestive Diseases Foundation, by grants from the University of Bari (Italy), Italian Association for Cancer Research (AIRC, Milan, Italy), by ERC-STG-IDEAS and by European Community's Seventh Framework Programme FP7/2007–20013 under grant Agreement No. 202272 (LipidomicNet). A. Morgano is a fellow of the G. d'Annunzio University Oncology Program. G.Lo Sasso is a fellow of AIRC (Milan, Italy). M. Petruzzelli is supported by Rosario Samanin Fund.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.03.021.

References

[1] Grundy, S.M. (1983) Absorption and metabolism of dietary cholesterol. Annu. Rev. Nutr. 3, 71–96.
[2] Altmann, S.W., Davis Jr., H.R., Zhu, L.J., Yao, X., Hoos, L.M., Tetzloff, G., Iyer, S.P., Maguire, M., Golovko, A., Zeng, M., Wang, L., Murgolo, N. and Graziano, M.P. (2004) Niemann-Pick C1 like 1 protein is critical for intestinal cholesterol absorption. Science 303, 1201–1204.
[3] Berge, K.E., Tian, H., Graf, G.A., Yu, L., Grishin, N.V., Schultz, J., Kwiterovich, P., Shan, B., Barnes, R. and Hobbs, H.H. (2000) Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 290, 1771–1775.
[4] Iqbal, J. and Hussain, M.M. (2005) Evidence for multiple complementary pathways for efficient cholesterol absorption in mice. J. Lipid Res. 46, 1491–1501.
[5] Brunham, L.R., Kruit, J.K., Iqbal, J., Fievet, C., Timmins, J.M., Pape, T.D., Coburn, B.A., Bissada, N., Staels, B., Groen, A.K., Hussain, M.M., Parks, J.S., Kuipers, F. and Hayden, M.R. (2006) Intestinal ABCA1 directly contributes to HDL biogenesis in vivo. J. Clin. Invest. 116, 1052–1062.
[6] Iqbal, J., Anwar, K. and Hussain, M.M. (2003) Multiple, independently regulated pathways of cholesterol transport across the intestinal epithelial cells. J. Biol. Chem. 278, 31610–31620.
[7] Mulligan, J.D., Flowers, M.T., Tebon, A., Bitgood, J.J., Wellington, C., Hayden, M.R. and Attie, A.D. (2003) ABCA1 is essential for efficient basolateral cholesterol efflux during the absorption of dietary cholesterol in chickens. J. Biol. Chem. 278, 13356–13366.
[8] Ohama, T., Hirano, K., Zhang, Z., Aoki, R., Tsujii, K., Nakagawa-Toyama, Y., Tsukamoto, K., Ikegami, C., Matsuyama, A., Ishigami, M., Sakai, N., Hiraoka, H., Ueda, K., Yamashita, S. and Matsuzawa, Y. (2002) Dominant expression of ATP-binding cassette transporter-1 on basolateral surface of Caco-2 cells stimulated by LXR/RXR ligands. Biochem. Biophys. Res. Commun. 296, 625–630.
[9] Repa, J.J., Buhman, K.K., Farese Jr., R.V., Dietschy, J.M. and Turley, S.D. (2004) ACAT2 deficiency limits cholesterol absorption in the cholesterol-fed mouse: impact on hepatic cholesterol homeostasis. Hepatology 40, 1088–1097.

[10] Wellington, C.L., Walker, E.K., Suarez, A., Kwok, A., Bissada, N., Singaraja, R., Yang, Y.Z., Zhang, L.H., James, E., Wilson, J.E., Francone, O., McManus, B.M. and Hayden, M.R. (2002) ABCA1 mRNA and protein distribution patterns predict multiple different roles and levels of regulation. Lab. Invest. 82, 273–283.
[11] Wang, N., Lan, D., Chen, W., Matsuura, F. and Tall, A.R. (2004) ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. Proc. Natl. Acad. Sci. USA 101, 9774–9779.
[12] Wang, N., Ranalletta, M., Matsuura, F., Peng, F. and Tall, A.R. (2006) LXR-induced redistribution of ABCG1 to plasma membrane in macrophages enhances cholesterol mass efflux to HDL. Arterioscler. Thromb. Vasc. Biol. 26, 1310–1316.
[13] Field, F.J., Born, E. and Mathur, S.N. (2004) Stanol esters decrease plasma cholesterol independently of intestinal ABC sterol transporters and Niemann-Pick C1-like 1 protein gene expression. J. Lipid Res. 45, 2252–2259.
[14] Murthy, S., Born, E., Mathur, S.N. and Field, F.J. (2002) LXR/RXR activation enhances basolateral efflux of cholesterol in CaCo-2 cells. J. Lipid Res. 43, 1054–1064.
[15] Repa, J.J. and Mangelsdorf, D.J. (2002) The liver X receptor gene team: potential new players in atherosclerosis. Nat. Med. 8, 1243–1248.
[16] Wang, D.Q. and Carey, M.C. (1996) Complete mapping of crystallization pathways during cholesterol precipitation from model bile: influence of physical–chemical variables of pathophysiologic relevance and identification of a stable liquid crystalline state in cold, dilute and hydrophilic bile salt-containing systems. J. Lipid Res. 37, 606–630.
[17] van Erpecum, K.J. and Carey, M.C. (1997) Influence of bile salts on molecular interactions between sphingomyelin and cholesterol: relevance to bile formation and stability. Biochim. Biophys. Acta 1345, 269–282.
[18] Smit, J.J., Schinkel, A.H., Oude Elferink, R.P., Groen, A.K., Wagenaar, E., van, D.L., Mol, C.A., Ottenhoff, R., van der Lugt, N.M., van Roon, M.A., et al. (1993) Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. Cell 75, 451–462.
[19] Voshol, P.J., Havinga, R., Wolters, H., Ottenhoff, R., Princen, H.M., Oude Elferink, R.P., Groen, A.K. and Kuipers, F. (1998) Reduced plasma cholesterol and increased fecal sterol loss in multidrug resistance gene 2 P-glycoprotein-deficient mice. Gastroenterology 114, 1024–1034.
[20] Eckhardt, E.R., Wang, D.Q., Donovan, J.M. and Carey, M.C. (2002) Dietary sphingomyelin suppresses intestinal cholesterol absorption by decreasing thermodynamic activity of cholesterol monomers. Gastroenterology 122, 948–956.
[21] Homan, R. and Hamelehle, K.L. (1998) Phospholipase A2 relieves phosphatidylcholine inhibition of micellar cholesterol absorption and transport by human intestinal cell line Caco-2. J. Lipid Res. 39, 1197–1209.
[22] Mackay, K., Starr, J.R., Lawn, R.M. and Ellsworth, J.L. (1997) Phosphatidylcholine hydrolysis is required for pancreatic cholesterol esterase- and phospholipase A2-facilitated cholesterol uptake into intestinal Caco-2 cells. J. Biol. Chem. 272, 13380–13389.
[23] Richmond, B.L., Boileau, A.C., Zheng, S., Huggins, K.W., Granholm, N.A., Tso, P. and Hui, D.Y. (2001) Compensatory phospholipid digestion is required for cholesterol absorption in pancreatic phospholipase A(2)-deficient mice. Gastroenterology 120, 1193–1202.
[24] Hay, D.W., Cahalane, M.J., Timofeyeva, N. and Carey, M.C. (1993) Molecular species of lecithins in human gallbladder bile. J. Lipid Res. 34, 759–768.
[25] van Ooteghem, N.A., Moschetta, A., Rehfeld, J.F., Samsom, M., van Erpecum, K.J. and van Berge-Henegouwen, G.P. (2002) Intraduodenal conjugated bile salts exert negative feedback control on gall bladder emptying in the fasting state without affecting cholecystokinin release or antroduodenal motility. Gut 50, 669–674.
[26] Nyberg, L., Duan, R.D. and Nilsson, A. (2000) A mutual inhibitory effect on absorption of sphingomyelin and cholesterol. J. Nutr. Biochem. 11, 244–249.
[27] Demel, R.A., Jansen, J.W., van Dijck, P.W. and van Deenen, L.L. (1977) The preferential interaction of cholesterol with different classes of phospholipids. Biochim. Biophys. Acta 465, 1–10.
[28] Field, F.J., Watt, K. and Mathur, S.N. (2008) Origins of intestinal ABCA1-mediated HDL–cholesterol. J. Lipid Res. 49, 2605–2619.
[29] Fromm, H., Amin, P., Klein, H. and Kupke, I. (1980) Use of a simple enzymatic assay for cholesterol analysis in human bile. J. Lipid Res. 21, 259–261.
[30] Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.
[31] Thomas, M.A., Lichtenstein, D.L., Krajcsi, P. and Wold, W.S. (2007) A real-time PCR method to rapidly titer adenovirus stocks. Methods Mol. Med. 130, 185–192.
[32] Moschetta, A., Vanberge-Henegouwen, G.P., Portincasa, P., Palasciano, G., Groen, A.K. and van Erpecum, K.J. (2000) Sphingomyelin exhibits greatly enhanced protection compared with egg yolk phosphatidylcholine against detergent bile salts. J. Lipid Res. 41, 916–924.
[33] Velardi, A.L., Groen, A.K., Elferink, R.P., van der, M.R., Palasciano, G. and Tytgat, G.N. (1991) Cell type-dependent effect of phospholipid and cholesterol on bile salt cytotoxicity. Gastroenterology 101, 457–464.
[34] Field, F.J., Chen, H., Born, E., Dixon, B. and Mathur, S. (1993) Release of ceramide after membrane sphingomyelin hydrolysis decreases the basolateral secretion of triacylglycerol and apolipoprotein B in cultured human intestinal cells. J. Clin. Invest. 92, 2609–2619.
[35] Repa, J.J., Turley, S.D., Lobaccaro, J.A., Medina, J., Li, L., Lustig, K., Shan, B., Heyman, R.A., Dietschy, J.M. and Mangelsdorf, D.J. (2000) Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. Science 289, 1524–1529.