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Inactivation of lipoprotein lipase occurs on the surface of THP-1 macrophages where oligomers of angiopoietin-like protein 4 are formed

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ABSTRACT

Lipoprotein lipase (LPL) hydrolyzes triglycerides in plasma lipoproteins causing release of fatty acids for metabolic purposes in muscles and adipose tissue. LPL in macrophages in the artery wall may, however, promote foam cell formation and atherosclerosis. Angiopoietin-like protein (ANGPTL) 4 inactivates LPL and *ANGPTL4* expression is controlled by peroxisome proliferator-activated receptors (PPAR). The mechanisms for inactivation of LPL by ANGPTL4 was studied in THP-1 macrophages where active LPL is associated with cell surfaces in a heparin-releasable form, while LPL in the culture medium is mostly inactive. The PPARδ agonist GW501516 had no effect on LPL mRNA, but increased ANGPTL4 mRNA and caused a marked reduction of the heparin-releasable LPL activity concomitantly with accumulation of inactive, monomeric LPL in the medium.

Intracellular ANGPTL4 was monomeric, while dimers and tetramers of ANGPTL4 were present in the heparin-releasable fraction and medium. GW501516 caused an increase in the amount of ANGPTL4 oligomers on the cell surface that paralleled the decrease in LPL activity. Actinomycin D blocked the effects of GW501516 on ANGPTL4 oligomer formation and prevented the inactivation of LPL. Antibodies against ANGPTL4 interfered with the inactivation of LPL.

We conclude that inactivation of LPL in THP-1 macrophages primarily occurs on the cell surface where oligomers of ANGPTL4 are formed.

1. Introduction

Lipoprotein lipase (LPL) hydrolyzes triglycerides (TG) in plasma lipoproteins. This function is considered to be anti-atherogenic, because it contributes to a low-risk lipoprotein profile. LPL produced by macrophages can have pro-atherogenic effects via bridging of lipoproteins to cells and extracellular matrix, thus promoting retention of lipoproteins in the artery wall and foam cell formation [1,2]. Angiopoietin-like protein (ANGPTL) 3 and 4 control LPL activity [3–6]. ANGPTL4 is present in tissues and blood as monomers, homo-oligomers and fragments generated by proteolytic cleavage, an N-terminal coiled-coil domain (N-ANGPTL4) and a C-terminal fibrinogen-like domain (C-ANGPTL4) [6]. N-ANGPTL4 binds to heparin and inactivates LPL via conversion of active LPL dimers to inactive monomers [5,7,8]. There are indications that ANGPTL4 oligomers are necessary for the inactivation [9,10]. Loss of function mutations in ANGPTL4 are connected with low levels of plasma triglycerides, and some mutants interfere with oligomerization of ANGPTL4 [10].

THP-1 macrophages produce LPL and respond to activation of PPARs by agonists [11]. *ANGPTL4* is a peroxisome proliferator-activated receptor (PPAR) target gene and PPAR γ and δ are up-regulated in differentiated macrophages [12]. We have activated PPARδ by the agonist GW501516 to study the molecular mechanisms for inactivation of LPL by ANGPTL4 in THP-1 macrophages.

2. Materials and methods

2.1. Materials

RPMI-1640 medium, glutamine, gentamicin and fetal calf serum (FCS) were from Invitrogen (Camarillo, CA). Bovine serum albumin

Abbreviations: ANGPTL4, angiopoietin-like protein 4; BSA, bovine serum albumin; COX-2, cyclooxygenase-2; DMSO, dimethylsolfoxide; LPL, lipoprotein lipase; PBS, phosphate-buffered saline; 10 mM phosphate, 0.15 M NaCl, pH 7.4; PMA, phorbol 12-myristate 13-acetate; PPAR, peroxisome proliferator-activated receptor.

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(BSA), phorbol 12-myristate 13-acetate (PMA), actinomycin D, dexamethasone and dimethyl sulfoxide (DMSO) were from Sigma–Aldrich (St. Louis, MO). Heparin was from Lövens (Malmö, Sweden). The PPARδ agonist, GW501516 (2-methyl-4-(((4-methyl-2-(4-trifluoromethylphenyl)-1,3-thiazol-5-yl)-methyl) sulfanyl) phenoxy) acetic acid, was synthesized by Synthelec AB (Sweden). The PPARα agonist (GW647) and PPARγ agonist GW929 were kind gifts from Prof. B. Staels (Lille, France).

2.2. Experimental procedure

THP-1 monocytes were from ATCC (Rockville, MD) and differentiated to macrophages with PMA for 4 days [13]. The PMA was omitted 24 h before treatment with PPAR agonists. For Western blots, experiments were carried out without FCS.

Human mononuclear cells were isolated from blood of healthy donors by centrifugation in Ficoll-Paque [14], plated at density 1.5×10^6 cells/ml RPMI-1640 medium with 2 mM glutamine and 50 μg/ml gentamicin for attachment (1 h, 37 °C) and grown for 2 days in the medium with 10% (v/v) autologous serum.

Macrophages were incubated with 100 nM GW501516 [15] or vehicle (DMSO) at 37 °C. In some experiments pre-incubation with actinomycin D (2 μg/ml, 30 min at 37 °C) was included. At the indicated time points, the incubation media were collected on ice. The cells were then washed with heparin (100 IU/ml RPMI-0.2% BSA, 30 min at 4 °C), and both media and heparin-releasable fractions were used for analysis of LPL and ANGPTL4. The macrophages were dissolved either in 0.2 M NaOH for measurement of protein content [16], in the Protein Loading Buffer (Fermentas) for SDS–PAGE or in RTL-buffer (Qiagen Nordic, Sweden) with β-mercaptoethanol for analysis of mRNA (Supplemental Methods).

In experiments with antibodies, the cells were treated with 100 nM GW501516 or vehicle for 30 min at 37 °C, then incubation continued for 24 h without agonist. MaxPab mouse polyclonal antibody against full length human ANGPTL4 (H00051129-B01P, Abnova), goat polyclonal antibody against a peptide near the N-terminus of human ANGPTL4 (sc-34113, Santa Cruz Biotechnology) or mouse monoclonal antibody against human CD71 (MCA1148GA, Serotec) were added to the cells in fresh medium with 10% heat-inactivated FCS to a final concentration of 20 μg/ml, and the incubation was continued for 4 h at 37 °C.

2.3. LPL and ANGPTL4 analyses

LPL activity and mass were measured as described [13]. One milliunit corresponds to release of one nanomole fatty acids per minute at 25 °C.

Dimeric and monomeric forms of LPL were separated from 15 ml of conditioned media by heparin-Sepharose chromatography [5].

ANGPTL4 protein mass was measured using the DuoSet ELISA Development kit (DY3485, R&D Systems), with some modifications: (a) incubation with capture antibody was carried out for 4 h at 37 °C; (b) blocking of non-specific binding sites was run for 1.5–2 h; (c) standard and samples were applied in 1% BSA-0.1% Tween 20; (d) the plates were incubated with samples for 16–20 h at 4 °C; (e) Streptavidin-HRP was added for 30–40 min; (f) as a substrate, OPD (1,2-phenylenediamine dihydrochloride) from "Dako", Denmark (S2045), was used. The reaction was stopped with 0.5 M H_2SO_4 (100 μl/well).

For Western blots, protease inhibitors (Complete Mini, Roche Diagnostics, USA) were immediately added to collected media. Buffer for heparin wash and Protein Loading Buffer contained protease inhibitors. Aliquots of media and heparin-releasable fractions were precipitated on ice with 12.5% TCA for 3 h using 30 μg of human insulin (Sigma–Aldrich, USA) per ml of sample as a carrier. Sedimented proteins (15,000 g, 30 min at 4 °C) were washed with cold acetone and dissolved in the Protein Loading Buffer (Fermentas). The samples were heated for 15 min at 70 °C with or without Reducing Agent (Fermentas) and immediately applied on Tris–Glycine PAGEr® Precast Gels (Lonza). The proteins separated by SDS–PAGE were transferred to a Hybond™-C Extra nitrocellulose membrane (Amersham, UK) and blotted using the ECL Advance Western Blotting Detection system (Amersham, UK). ANGPTL4 in non-reduced samples was detected with the MaxPab mouse polyclonal antibody against full length human ANGPTL4 (H00051129-B01, Abnova, 1:3 000) followed by peroxidase labeled goat polyclonal antibody against mouse IgG (A2554, Sigma, 1:25 000). ANGPTL4 in reduced samples was detected with the goat polyclonal antibody against a peptide near the N-terminus of human ANGPTL4 (sc-34113, Santa Cruz Biotechnology, 1:3000) followed by peroxidase labeled rabbit polyclonal antibody against goat immunoglobulins (P0449, DakoCytomation, 1:10,000).

2.4. Statistics

Statistical analysis of the data was performed using unpaired Student's *t* test.

3. Results

With macrophages derived from THP-1 monocytes, most of the LPL is found in the culture medium [17]. In our experiments (after 24 h culture), only 5–10% of the total LPL mass in the system was released by heparin from the cell surfaces and less than 3% remained with the cells. The heparin-releasable LPL had high specific activity (activity/mass), 170–200 mU/μg LPL, indicating that it represented newly synthesized, mostly active LPL. The specific activity of LPL in the medium was only 20–25 mU/μg. For analysis of the mechanisms for the inactivation of LPL, and the possible involvment of ANGPTL4, THP-1 macrophages were treated with different PPAR agonists. The PPARδ agonist GW501516 had the strongest effect (Supplemental Fig. S1), and was selected for further studies. Activation of PPARδ by GW501516 was confirmed by expression of COX-2 protein [15] without increase in PPARδ mRNA level (Supplemental Fig. S2).

3.1. Time courses for the effect of GW501516 on LPL activity and ANGPTL4 expression and oligomerization

To study the inactivation process THP-1 macrophages were incubated for different times with the PPARδ agonist GW501516 or vehicle (Fig. 1). Significantly more LPL protein was found in the 24 h-conditioned medium from GW501516-treated cells (Fig. 1A). LPL activity in the medium increased during the first 3 h in both groups. Then a steady state for LPL activity was reached with the control cells between secretion of new, active LPL and loss of LPL activity, while with the agonist-treated cells a rapid loss of LPL activity had started (Fig. 1B). Significant loss of both LPL mass (Fig. 1C) and activity (Fig. 1D) in the heparin-releasable fraction was detected from 6 h for cells with GW501516, compared to cells with vehicle only. The loss was not due to a reduced number of LPL binding sites on the cell surfaces (experiments with ^{125}I-labeled bovine LPL, data not shown). The level of ANGPTL4 mRNA was up-regulated already after 3 h with GW501516, reached a maximum at 6 h, and remained high to the end of the experiment (24 h, Fig. 1E). In contrast, the levels of LPL mRNA did not change during the experiment, indicating that the effect of GW501516 on LPL was post-transcriptional (Fig. 1F).

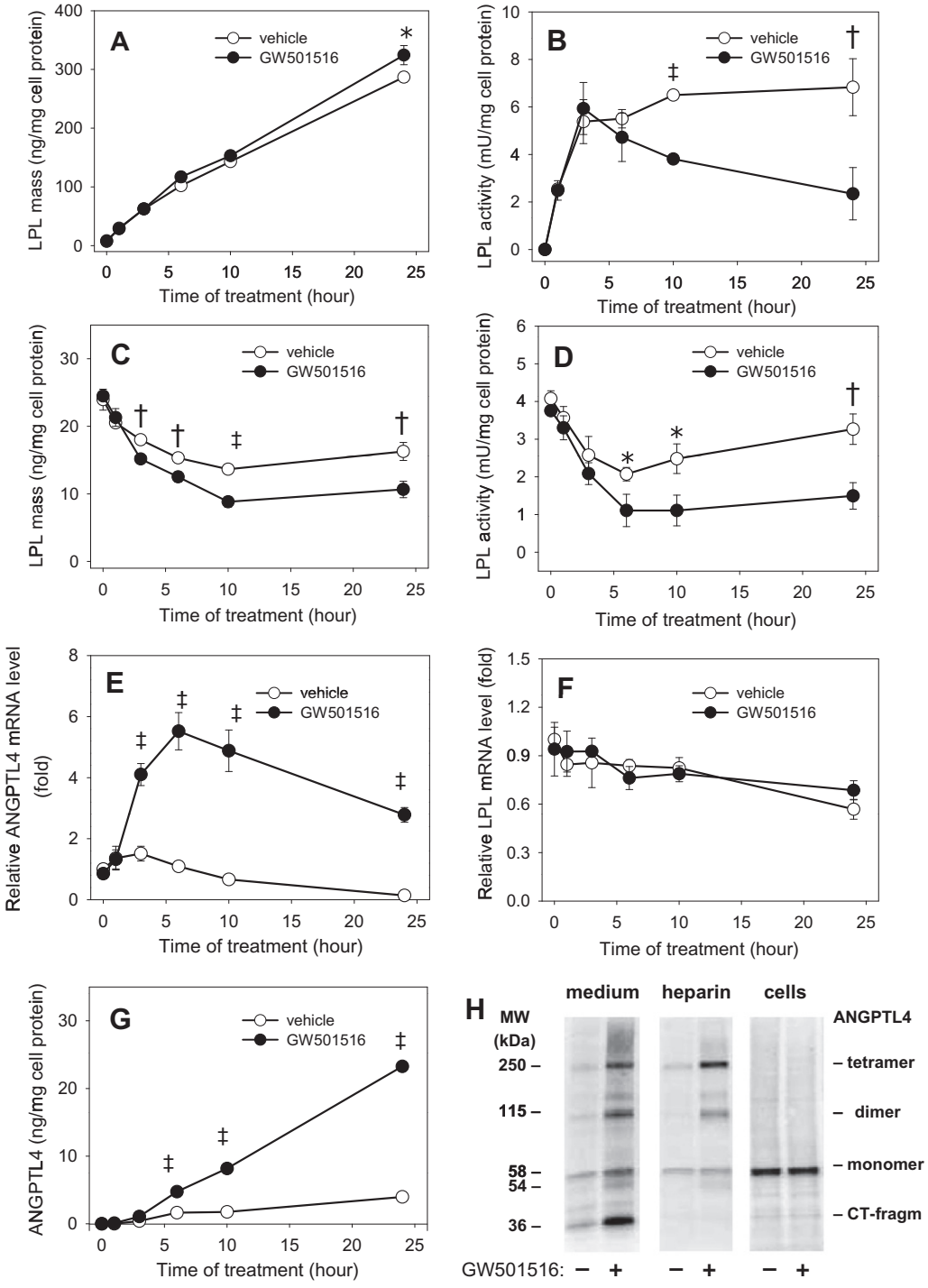

Fig. 1. Effect of GW501516 on LPL and ANGPTL4 in THP-1 macrophages. Cells were incubated with vehicle only (*open* circles) or with 100 nM GW501516 (*solid* circles) for the indicated time and then washed with heparin (100 IU/ml, 30 min at 4 °C). (A) LPL protein and (B) LPL activity in medium; (C) LPL protein and (D) LPL activity in heparin wash; (E) ANGPTL4 mRNA and (F) LPL mRNA; (G) ANGPTL4 protein in medium. mRNA was normalized to β-actin mRNA and presented relative to the mean value obtained for untreated cells at time 0 h. The data are means of values from three wells ± S.D.*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ (GW501516 vs vehicle). (H) ANGPTL4 pattern on Western blots (SDS–PAGE, non-reducing conditions) of medium, heparin wash and remaining cells after 24 h-incubation with either vehicle or 100 nM GW501516. Antibody against full length human ANGPTL4 was used. Samples applied to the lanes corresponded to 65 (*medium*), 170 (*heparin*) and 20 (*cells*) μg of total cell protein, respectively.

ANGPTL4 protein in the medium increased continuously from 6 h to the end of experiment when it was several times higher with GW 501516-treated cells than with control cells (Fig. 1G). Incubation of THP-1 macrophages with antibodies against ANGPTL4 (N-15, B01P), but not with an irrelevant antibody (anti-CD71), prevented the GW501516-dependent loss of LPL activity both in medium and in the heparin-releasable fraction (Fig. 2).

Analysis of 24 h-conditioned media on heparin-Sepharose showed conversion of active dimeric LPL to inactive monomers after treatment with GW501516 (Supplemental Fig. S3), similar to the effect of N-terminal ANGPTL4 on bovine LPL *in vitro* [5]. ANGPTL4 eluted before the LPL monomer peak, illustrating that ANGPTL4 did not remain bound to any of the forms of LPL.

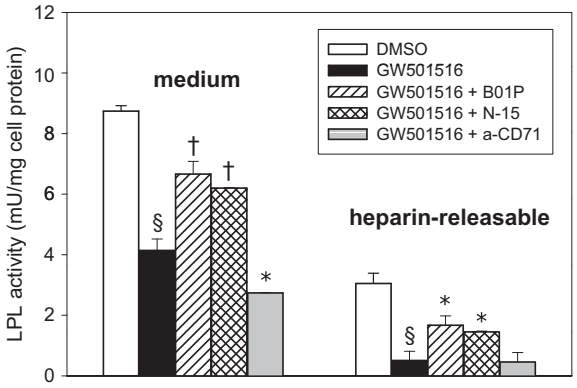

Fig. 2. Antibodies to human ANGPTL4 prevented the GW501516-induced inactivation of LPL. Cells, first stimulated or not with 100 nM GW501516, were incubated for 4 h with/without antibodies. Medium and heparin wash (100 IU/ml, 30 min at 4 °C) were collected for analysis of LPL activity. Data are means of values from three wells ± S.D. $^{§}p < 0.01$ (GW501516 vs vehicle); $^{*}p < 0.05$, $^{†}p < 0.01$ (GW501516 + antibody vs GW501516 only).

3.2. GW501516 stimulates formation of ANGPTL4 oligomers on cell surfaces

ANGPTL4 was analyzed by Western blot of non-reduced samples. In heparin-washed cells only monomers of ANGPTL4 (58 kDa) were found. In contrast, dimers (115 kDa) and tetramers (around 250 kDa), were present both in media and in the heparin-releasable fractions (Fig. 1H). A C-terminal 36 kDa-fragment was detected only in media (no reaction with antibodies against the N-terminal part of ANGPTL4). Scanning revealed that after GW501516-treatment tetramers and dimers in the heparin-releasable fraction increased 5- and 7-fold, respectively, compared to control cells, while monomers increased only 1.5-fold. In medium tetramers, dimers, monomers and the 36 kDa-fragment increased 3-, 4-, 2- and 3.5-fold, respectively. In contrast, ANGPTL4 in the cells (always monomers) was not affected by GW501516.

3.3. Changes in ANGPTL4 oligomer patterns in relation to inactivation of LPL

To study the relation between oligomerization of ANGPTL4 on one side, and inactivation of LPL on the other, the cells were washed with heparin (100 IU/ml, 20 min at 37 °C) to remove LPL and ANGPTL4 from the cell surfaces. The cells were then incubated with agonist or vehicle (without FCS) to follow LPL and ANGPTL4 as they appeared on the plasma membrane (Supplemental Fig. S4). As in experiments with FCS (Fig. 1), treatment with GW501516 resulted in accumulation of LPL with low activity (Fig. S4, A and B) and in increased ANGPTL4 in the medium(Fig. S4C) and (Fig. S4D). SDS–PAGE under non-reducing conditions (Fig. 3A and B) demonstrated increased amounts of ANGPTL4 oligomers in the heparin-releasable fraction and in the medium, starting at 6 h of GW501516 treatment, compared to control cells. These changes paralleled the loss of LPL activity in medium and in the heparin-releasable fraction (Fig. S4B; Fig. 1, B and D).

It was previously known that mRNA for ANGPTL4 is relatively short-lived, while that of LPL is more stable [5]. In accordance, treatment of THP-1 macrophages with the transcription blocker actinomycin D before GW501516 prevented the elevation of ANGPTL4 mRNA, but not LPL mRNA, and completely blocked the increase of ANGPTL4 in the media (Supplemental Table 1) without changes in the intracellular pool of ANGPTL4 (Fig. 3D, lanes 7 and 8 for technical reasons got only 44% cell extract compared to lanes 5 and 6). Almost no ANGPTL4 oligomers were found in the heparin-releasable fractions after treatment with actinomycin D (Fig. 3C) and no inactivation of LPL induced by GW501516 was detected (at 3, 6 and 10 h of the LPL activities in the medium were 1.07 ± 0.096, 1.17 ± 0.030 and 1.31 ± 0.001 mU/ml, respectively).

3.4. Analyses of inactivation of LPL by ANGPTL4 in the incubation medium

Inactivation of both human LPL and bovine LPL *in vitro* in 24 h-conditioned media (Supplemental Fig. S5) demonstrated that, despite that concentrations of ANGPTL4 in the conditioned media

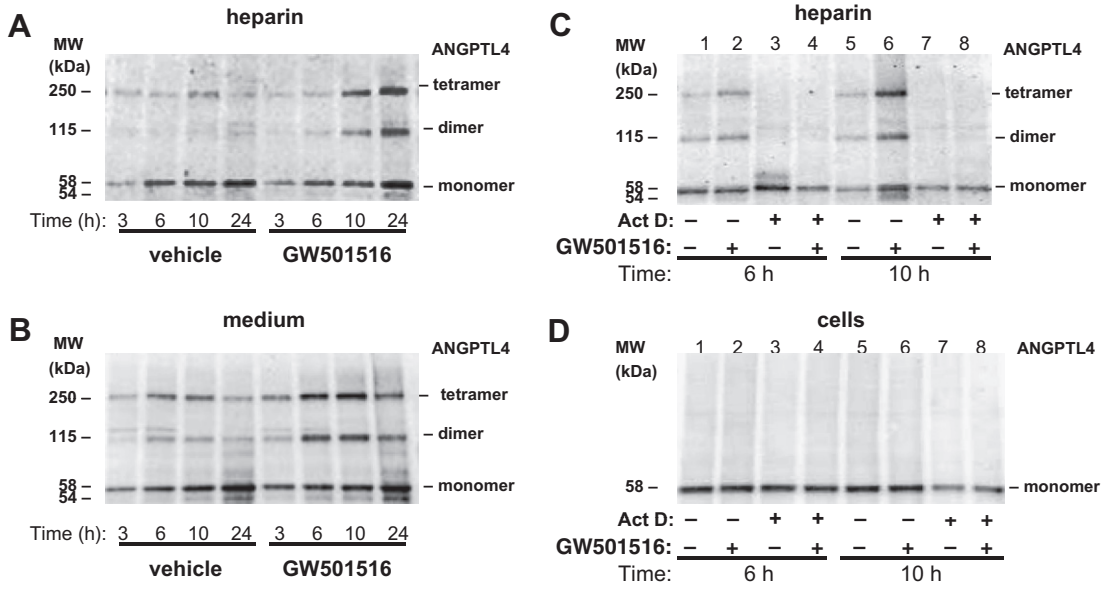

Fig. 3. Effects of GW501516 and actinomycin D on oligomerization of ANGPTL4. Cells were pre-incubated with or without actinomycin D for 30 min at 37 °C (C and D) and incubated with vehicle only or with 100 nM GW501516 for the indicated time. ANGPTL4 pattern in samples of media, heparin wash (100 IU/ml, 30 min at 4 °C) and cells was analyzed by Western blot as described in Fig. 1. (A and B) Samples were obtained after incubation of the same numbers of cells. (C and D) For technical reasons the lanes represent samples from different amounts of total cell protein: for *heparin*: lanes 1,2,5 and 6 – from 95 μg, lanes 3 and 4 – from 60 μg and lannes 7 and 8– from 45 μg; for *cells:* lanes 1,2,5 and 6 – from 45 μg, lanes 3 and 4 – from 30 μg and lanes 7 and from 8 – from 20 μg.

from agonist-treated cells reached 20–25 ng/ml (detected by ELISA) and exceeded those in conditioned media from control cells by a factor of 20–50, no marked difference was seen in the rate of LPL inactivation in medium with (Fig. S5A) or without (Fig. S5B) FCS. Similar results were obtained with bovine LPL (Fig. S5C). Addition of recombinant human N-terminal ANGPTL4 (N-ANGPTL4) to heparin-washed THP-1 macrophages (10 ng/ml medium) resulted in complete loss of LPL activity after 1 h of incubation, while a ten-fold lower concentration was not enough for full inactivation.

3.5. GW501516 has similar effects on LPL and ANGPTL4 in macrophages derived from human blood monocytes

In blood monocytes the low levels of LPL mRNA increased in macrophages, while the relatively high levels of ANGPTL4 mRNA decreased with differentiation (Supplemental Table 2). Treatment for 24 h with GW501516 increased ANGPTL4 mRNA and decreased LPL activity in the media (compared to cells treated with vehicle only), while LPL mRNA and protein in the media were relatively unaffected. This was similar to the effects demonstrated with THP-1 macrophages. Because the blood monocyte-derived macrophages needed serum in the incubation medium at all times, we were unable to study the oligomerization of ANGPTL4 in this system.

4. Discussion

Modulation of LPL activity by ANGPTL4 has been demonstrated before in other systems [3–6,10,18,19]. We have used THP-1 macrophages for detailed studies of where and when inactivation of LPL by ANGPTL4 occurs in a cell system. Differentiation of THP-1 cells to macrophages with PMA increases the expression of PPARδ [12], making them more responsive to specific agonists. The expression of ANGPTL4 was very low in the basal state and increased several-fold within a few hours from addition of the agonist GW501516. Moreover, the THP-1 cells could stand incubation in serum-free medium, necessary for studies of the oligomer states of ANGPTL4 by Western blots. Both ANGPTL4 and LPL are PPAR target genes [20–22]. The present data show that the response to GW501516 is rapid for ANGPTL4 mRNA, but not for LPL mRNA, and that increased ANGPTL4 expression coincided with decreased LPL activity. Two different antibodies to ANGPTL4 were shown to counteract the PPARδ-induced inactivation of LPL, demonstrating that ANGPTL4 was involved in control of LPL activity.

Inactivation of LPL occurs via dissociation of active LPL homodimers to inactive monomers [23]. Previous *in vitro* experiments demonstrated that the N-terminal part of ANGPTL4 binds to LPL and causes dissociation of the dimers [5]. In concert with this, separation of conditioned media from THP-1 macrophages on heparin-Sepharose demonstrated a shift of LPL from active dimers to inactive monomers after treatment of the cells with GW501516. This coincided with a several-fold increase in ANGPTL4 protein both in the medium and in the heparin-releasable fraction.

The cell surface-bound LPL activity represents a small fraction of the total LPL activity in THP-1 macrophages. This fraction decreased significantly after treatment with GW501516, starting from 3 h, when more ANGPTL4 had appeared on the cell surface. The amounts of ANGPTL4 and of inactive LPL in the medium increased thereafter, indicating that inactivation of LPL occurred on the cell surfaces. Experiments with conditioned media confirmed that inactivation of LPL by ANGPTL4 present in the medium was unlikely. ANGPTL4 on the cell surfaces was barely detectable by Western blots, but was sufficient for inactivation of newly synthesized LPL. The two-dimensional concentrations of LPL and ANGPTL4 on the cell surface, and binding to heparin-like structures, may promote the inactivation and release of inactive LPL to the medium. LPL monomers have lower affinity to heparin and heparan sulfate than LPL dimers [24]. In the THP-1 system the amount of ANGPTL4 in the medium was much higher than that on the cell surface, while most of the ANGPTL4 protein was found within the cells. Our data demonstrate that inactivation of LPL by ANGPTL4 occurred on the cell surfaces where both proteins stay before they are released into the medium. When ANGPTL4 was not up-regulated, more active LPL was released to the medium. The release of LPL is probably due to shedding of cell surface glycan chains by the macrophages [25].

Formation of covalent oligomers of ANGPTL4 on the plasma membranes of THP-1 macrophages coincided in time with inactivation of LPL. The importance of ANGPTL4 oligomerization for inactivation of LPL was previously proposed [9,10]. It was demonstrated that oligomerization is an inherent property of the ANGPTL4 protein, and that inter-chain disulfide bonds are not needed [8].

The heparin affinity of ANGPTL4 resides in the N-terminal part [5]. Recombinant N-terminal domain binds to the extracellular matrix, while the C-terminal part is released into media [26]. Cleavage of ANGPTL4 depends on serum factors [9] and reduces the inhibitory effect of ANGPTL4 on LPL activity [27]. We found increased amounts of C-ANGPTL4 in media of THP-1 macrophages after stimulation with GW501516. No fragment corresponding to N-ANGPTL4 was found in any of the compartments, indicating that this fragment was rapidly degraded. Because serum was omitted in these experiments, the cells themselves must have produced the proteolytic activity. A similar conclusion was reached in studies of ANGPTL4 transfected into human embryonic kidney cells [10].

The changes in ANGPTL4 expression and oligomerization, induced by the PPARδ agonist and actinomycin D, coincided with modulations of LPL activity. The lack of ANGPTL4 oligomers on the surfaces of cells treated with actinomycin D indicated that oligomerization of ANGPTL4 is controlled by yet another gene product, that also has a short-lived, and possibly PPAR-dependent, mRNA.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.07.048.

References

[1] I.J. Goldberg, Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis, J. Lipid Res. 37 (1996) 693–707.
[2] M.O. Pentikainen, R. Oksjoki, K. Oorni, P.T. Kovanen, Lipoprotein lipase in the arterial wall: linking LDL to the arterial extracellular matrix and much more, Arterioscler. Thromb. Vasc. Biol. 22 (2002) 211–217.
[3] K. Yoshida, T. Shimizugawa, M. Ono, H. Furukawa, Angiopoietin-like protein 4 is a potent hyperlipidemia-inducing factor in mice and inhibitor of lipoprotein lipase, J. Lipid Res. 43 (2002) 1770–1772.
[4] S. Mandard, F. Zandbergen, N.S. Tan, P. Escher, D. Patsouris, W. Koenig, R. Kleemann, A. Bakker, F. Veenman, W. Wahli, M. Muller, S. Kersten, The direct peroxisome proliferator-activated receptor target fasting-induced adipose factor (FIAF/PGAR/ANGPTL4) is present in blood plasma as a truncated protein that is increased by fenofibrate treatment, J. Biol. Chem. 279 (2004) 34411–34420.
[5] V. Sukonina, A. Lookene, T. Olivecrona, G. Olivecrona, Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue, Proc. Natl. Acad. Sci. USA 103 (2006) 17450–17455.
[6] F. Mattijssen, S. Kersten, Regulation of triglyceride metabolism by Angiopoietin-like proteins, Biochim. Biophys. Acta 2012 (1821) 782–789.

[7] M.H. Yau, Y. Wang, K.S. Lam, J. Zhang, D. Wu, A. Xu, A highly conserved motif within the NH2-terminal coiled-coil domain of angiopoietin-like protein 4 confers its inhibitory effects on lipoprotein lipase by disrupting the enzyme dimerization, J. Biol. Chem. 284 (2009) 11942–11952.
[8] L. Shan, X.C. Yu, Z. Liu, Y. Hu, L.T. Sturgis, M.L. Miranda, Q. Liu, The angiopoietin-like proteins ANGPTL3 and ANGPTL4 inhibit lipoprotein lipase activity through distinct mechanisms, J. Biol. Chem. 284 (2009) 1419–1424.
[9] H. Ge, G. Yang, L. Huang, D.L. Motola, T. Pourbahrami, C. Li, Oligomerization and regulated proteolytic processing of angiopoietin-like protein 4, J. Biol. Chem. 279 (2004) 2038–2045.
[10] W. Yin, S. Romeo, S. Chang, N.V. Grishin, H.H. Hobbs, J.C. Cohen, Genetic variation in ANGPTL4 provides insights into protein processing and function, J. Biol. Chem. 284 (2009) 13213–13222.
[11] M. Ogata, M. Tsujita, M.A. Hossain, N. Akita, F.J. Gonzalez, B. Staels, S. Suzuki, T. Fukutomi, G. Kimura, S. Yokoyama, On the mechanism for PPAR agonists to enhance ABCA1 gene expression, Atherosclerosis 205 (2009) 413–419.
[12] H. Vosper, G.A. Khoudoli, C.N. Palmer, The peroxisome proliferator activated receptor delta is required for the differentiation of THP-1 monocytic cells by phorbol ester, Nucl. Recept. 1 (2003) 9.
[13] E. Makoveichuk, S. Castel, S. Vilaro, G. Olivecrona, Lipoprotein lipase-dependent binding and uptake of low density lipoproteins by THP-1 monocytes and macrophages: possible involvement of lipid rafts, Biochim. Biophys. Acta 1686 (2004) 37–49.
[14] D. Milosavljevic, A. Kontush, S. Griglio, G. Le Naour, J. Thillet, M.J. Chapman, VLDL-induced triglyceride accumulation in human macrophages is mediated by modulation of LPL lipolytic activity in the absence of change in LPL mass, Biochim. Biophys. Acta 1631 (2003) 51–60.
[15] B. Glinghammar, J. Skogsberg, A. Hamsten, E. Ehrenborg, PPARdelta activation induces COX-2 gene expression and cell proliferation in human hepatocellular carcinoma cells, Biochem. Biophys. Res. Commun. 308 (2003) 361–368.
[16] M.A. Markwell, S.M. Haas, L.L. Bieber, N.E. Tolbert, A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples, Anal. Biochem. 87 (1978) 206–210.
[17] J.H. Auwerx, S. Deeb, J.D. Brunzell, R. Peng, A. Chait, Transcriptional activation of the lipoprotein lipase and apolipoprotein E genes accompanies differentiation in some human macrophage-like cell lines, Biochemistry 27 (1988) 2651–2655.
[18] A. Koster, Y.B. Chao, M. Mosior, A. Ford, P.A. Gonzalez-DeWhitt, J.E. Hale, D. Li, Y. Qiu, C.C. Fraser, D.D. Yang, J.G. Heuer, S.R. Jaskunas, P. Eacho, Transgenic angiopoietin-like (angptl)4 overexpression and targeted disruption of angptl4 and angptl3: regulation of triglyceride metabolism, Endocrinology 146 (2005) 4943–4950.
[19] H. Staiger, C. Haas, J. Machann, R. Werner, M. Weisser, F. Schick, F. Machicao, N. Stefan, A. Fritsche, H.U. Haring, Muscle-derived angiopoietin-like protein 4 is induced by fatty acids via peroxisome proliferator-activated receptor (PPAR)-delta and is of metabolic relevance in humans, Diabetes 58 (2009) 579–589.
[20] S. Kersten, S. Mandard, N.S. Tan, P. Escher, D. Metzger, P. Chambon, F.J. Gonzalez, B. Desvergne, W. Wahli, Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene, J. Biol. Chem. 275 (2000) 28488–28493.
[21] J.C. Yoon, T.W. Chickering, E.D. Rosen, B. Dussault, Y. Qin, A. Soukas, J.M. Friedman, W.E. Holmes, B.M. Spiegelman, Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation, Mol. Cell Biol. 20 (2000) 5343–5349.
[22] K. Schoonjans, J. Peinado-Onsurbe, A.M. Lefebvre, R.A. Heyman, M. Briggs, S. Deeb, B. Staels, J. Auwerx, PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene, Embo J. 15 (1996) 5336–5348.
[23] J.C. Osborne Jr., G. Bengtsson-Olivecrona, N.S. Lee, T. Olivecrona, Studies on inactivation of lipoprotein lipase: role of the dimer to monomer dissociation, Biochemistry 24 (1985) 5606–5611.
[24] A. Lookene, O. Chevreuil, P. Ostergaard, G. Olivecrona, Interaction of lipoprotein lipase with heparin fragments and with heparan sulfate: stoichiometry, stabilization, and kinetics, Biochemistry 35 (1996) 12155–12163.
[25] V. Kainulainen, H. Wang, C. Schick, M. Bernfield, Syndecans, heparan sulfate proteoglycans, maintain the proteolytic balance of acute wound fluids, J. Biol. Chem. 273 (1998) 11563–11569.
[26] C. Chomel, A. Cazes, C. Faye, M. Bignon, E. Gomez, C. Ardidie-Robouant, A. Barret, S. Ricard-Blum, L. Muller, S. Germain, C. Monnot, Interaction of the coiled-coil domain with glycosaminoglycans protects angiopoietin-like 4 from proteolysis and regulates its antiangiogenic activity, Faseb J. 23 (2009) 940–949.
[27] X. Lei, F. Shi, D. Basu, A. Huq, S. Routhier, R. Day, W. Jin, Proteolytic processing of angiopoietin-like protein 4 by proprotein convertases modulates its inhibitory effects on lipoprotein lipase activity, J. Biol. Chem. 286 (2011) 15747–15756.