FEBS Letters 588 (2014) 65–70

FEBS Letters

journal homepage: www.FEBSLetters.org

Macrophage mitochondrial damage from StAR transport of 7-hydroperoxycholesterol: Implications for oxidative stress-impaired reverse cholesterol transport

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ARTICLE INFO

Article history:
Received 27 August 2013
Revised 16 October 2013
Accepted 25 October 2013
Available online 21 November 2013

Edited by Laszlo Nagy

Keywords:
Oxidative stress
Cholesterol hydroperoxide
StAR protein
Macrophage
Reverse cholesterol transport

ABSTRACT

StAR family proteins in vascular macrophages participate in reverse cholesterol transport (RCT). We hypothesize that under pathophysiological oxidative stress, StARs will transport not only cholesterol to macrophage mitochondria, but also pro-oxidant cholesterol hydroperoxides (7-OOHs), thereby impairing early-stage RCT. Upon stimulation with dibutyryl-cAMP, RAW264.7 macrophages exhibited a strong time-dependent induction of mitochondrial StarD1 and plasma membrane ABCA1, which exports cholesterol. 7α-OOH uptake by stimulated RAW cell mitochondria (like cholesterol uptake) was strongly reduced by StarD1 knockdown, consistent with StarD1 involvement. Upon uptake by mitochondria, 7α-OOH (but not redox-inactive 7α-OH) triggered lipid peroxidation and membrane depolarization while reducing ABCA1 upregulation. These findings provide strong initial support for our hypothesis.

1. Introduction

Expression of scavenger receptors such as SR-B1 and CD36 by vascular macrophages is not regulated by cholesterol negative feedback [1]. Consequently, under conditions of oxidative stress, these cells could potentially become engorged with cholesterol and other lipids due to unregulated uptake of oxidized low density lipoprotein (oxLDL) [2]. Export to HDL is a key step in reverse cholesterol transport (RCT) by which macrophages limit or reverse deleterious lipid accumulation [3,4]. If export capacity is impaired or overwhelmed, macrophages can be transformed to lipid-laden "foam cells", which accumulate in the vascular wall and contribute to atherogenesis [2–4]. In addition to plasma membrane scavenger receptors, macrophages express other proteins that play crucial roles in inward/outward cholesterol trafficking. These include steroidogenic acute regulatory (StAR) family proteins, which bind preexisting or incoming cholesterol and deliver it to/into mitochondria for conversion to 27-hydroxycholesterol (27-OH) by the CYP27A1 system [5,6]. Most StAR proteins contain a START domain pocket which accommodates a single cholesterol molecule in highly specific fashion, i.e. with little (if any) recognition of non-sterol lipids [7]. 27-OH is a key agonist of liver-X-receptor (LXR) transcription factors, which, upon interacting with retinoid-X-receptor in the nucleus, induce expression of the plasma membrane ATP-binding cassette transporters ABCA1 and ABCG1 [8,9]. These proteins play crucial roles in exporting excess cholesterol to extracellular acceptors, ABCA1 sending it mainly to apoA1, the lipid-free/poor protein of HDL [8]. Cholesterol loading or 8-Br-cAMP treatment of mouse or human macrophages induces several regulatory proteins, including StarD1 and CYP27A1, resulting in 27-OH formation and elevated cholesterol efflux to re-establish homeostasis [10]. Mature StarD1, found in the mitochondrial outer membrane, participates in cholesterol transport to the inner membrane for processing by CYP27A1. StarD4, a structural homologue of StarD1 that lacks organelle-targeting sequences, probably functions as a cytosolic cholesterol transporter [6,11].

Abbreviations: ABCA1, ATP binding cassette transporter A1; C11-BODIPY, 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3*a*,4*a*-diaza-*s*-inda-cene-3-undecanoicacid; db-cAMP, dibutyryl-cAMP; ChOOH(s), cholesterol hydroperoxide(s); JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-benzimidazolylcarbocyanine iodide; MTT, 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide; 7α-OOH, 3β-hydroxycholest-5-ene-7α-hydroperoxide; 7α-OH, cholest-5-ene-3β,7α-diol; StarD1, type-1 steroidogenic acute regulatory domain protein; StarD4, type-4 steroidogenic acute regulatory domain protein; PBS, phosphate-buffered saline; POPC, 1-palmitoyl-2-*sn*-glycero-3-phosphocholine; SUV(s), small unilamellar vesicle(s)

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0014-5793/$36.00 © 2013 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
http://dx.doi.org/10.1016/j.febslet.2013.10.051

Free cholesterol in LDL and cell membranes under oxidative stress can be converted to various oxides, including redox-active hydroperoxides ChOOHs) such as 7α- and 7β-OOH [12]. Like all lipid hydroperoxides, ChOOHs are subject to (i) iron-catalyzed one-electron reduction to oxidizing free radicals or (ii) enzyme-catalyzed two-electron reduction to redox-inert alcohols [12]. If pathway (i) predominates, it may expand oxidative damage by triggering chain lipid peroxidation. Studies in this laboratory [13] have revealed that ChOOHs may also undergo spontaneous or transfer protein-enhanced translocation to membrane or lipoprotein acceptors, where processes (i) or (ii) may ensue. Translocation may result in the "broadcasting" of ChOOH toxicity or redox signaling [13]. In support of this idea, we showed previously that membrane depolarization of isolated mitochondria during exposure to 7α-OOH was enhanced by SCP-2, a non-specific lipid transfer protein [14]. 7α-OOH was subsequently shown to be more toxic to SCP-2-overexpressing cells than to controls, and this correlated with greater 7α-OOH uptake, one-electron turnover in mitochondria, and loss of membrane potential [15]. Turning more recently to sterol-specific StAR family proteins, we showed that recombinant StarD4 accelerated membrane-damaging transfer of 7α-OOH to isolated mitochondria, whereas it had no effect on phosphatidylcholine hydroperoxide transfer [16].

The goal of this study was to test the novel hypothesis that under oxidative stress associated, for example, with chronic obesity and atherogenesis, StAR proteins transport not only cholesterol to macrophage mitochondria, but also ChOOHs, which trigger site-specific peroxidative damage that impairs RCT at its early stages.

2. Materials and methods

2.1. General materials

Sigma–Aldrich (St. Louis, MO) supplied the cholesterol, db-cAMP, MTT, JC-1, and fetal bovine serum. C11-BODIPY was from Molecular Probes (Eugene, OR). POPC and 7α-OH were from Avanti Polar Lipids (Birmingham, AL). Boehringer Mannheim (Indianapolis, IN) supplied the Complete-Mini mixture of protease inhibitors. Primary antibodies against mouse StarD1, StarD4, ABCA1, and human β-actin were from Santa Cruz Biotechnology (Santa Cruz, CA). A horseradish peroxidase-conjugated IgG secondary antibody was from Cell Signaling Technology (Danvers, MA). Amersham Biosciences (Arlington Heights, IL) supplied the [4-^{14}C]cholesterol (~50 mCi/ml). 7α-OOH was prepared by dye-sensitized photooperoxidation of cholesterol, as described [17]; [^{14}C]7α-OOH was prepared similarly, using [4-^{14}C]cholesterol.

2.2. Cell culture, stimulation, and challenge

Mouse RAW 264.7 macrophages (ATCC) were cultured in humidified 5% CO_2, 95% air at 37 °C in DME medium supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (0.1 mg/ml). Prior to an experiment, cells at ~70% confluency were incubated for up to 16 h at 37 °C in DME medium that either contained 1.0 mM db-cAMP or lacked it. After this incubation, where indicated, cells were washed again and exposed to small (50-nm) unilamellar liposomes (SUVs) composed of POPC/cholesterol (1:1 by mol), POPC/cholesterol/7α-OH (1:0.8:0.2 by mol), or POPC/cholesterol/7α-OOH (1:0.8:0.2 by mol) in DME medium.

2.3. StarD1 knockdown

Silencer siRNAs for mouse StarD1 and scrambled oligonucleotide pairs were purchased from Ambion (Austin, TX). Single transfections of duplex mixtures were carried out in OPTI-MEM medium (Gibco/Life Technologies, Grand Island, NY) for 8 h, using X-tremeGENE (18 μl/ml) as advised by the supplier (Roche Diagnostics, Indianapolis, IN). Following transfection, cells were switched to full growth medium without siRNA or X-tremeGENE for a 36–48 h recovery period, then washed, overlaid with DME medium, and stimulated with 1 mM db-cAMP for 9–12 h. After this, they were either challenged with SUV-7α-OOH or scraped into ice-cold protease inhibitor-containing PBS and analyzed for StarD1, StarD4, and ABCA1 expression by Western blotting [18].

2.4. Determination of mitochondrial uptake of 7α-OOH

Mitochondrial uptake of 7α-OOH, was determined essentially as described [18]. Briefly, stimulated or non-stimulated wild type (WT) and knockdown (kd) cells were incubated in serum-free RPMI medium with 50 mM [^{14}C]7α-OOH in POPC/cholesterol/7α-OOH SUVs for up to 5 h. Following incubation, cells were washed, scraped into ice-cold PBS, and pelleted by centrifugation. Mitochondria were isolated by differential centrifugation [18], quantified by BCA protein assay, and subjected to scintillation counting.

2.5. Measurement of mitochondrial membrane potential ($\Delta\Psi_m$)

WT and kd cells in black-wall 96-well plates were either untreated or stimulated with 1 mM db-cAMP in DME medium for 9 h, and exposed to SUV-7α-OOH in either increasing concentrations (10–100 μM) for a fixed time (5 h), or 100 μM SUV-7α-OOH for increasing times up to 7 h. After peroxide exposure, cells were washed, incubated with JC-1 (5 μg/ml) for 30 min, washed again, and analyzed using a BioTek Synergy-MX fluorescence plate reader (Winooski, VT). Settings were as follows: red (λ_{ex} 560 nm; λ_{em} 595 nm); green (λ_{ex} 485 nm; λ_{em} 535 nm).

2.6. Assessment of 7α-OOH-induced lipid peroxidation and cell death

Cells grown on FlexiPERM slides (Sarstedt, Numbrecht, Germany) in glass-bottom dishes were stimulated for 9 h with 1 mM db-cAMP, then treated with 2 μM C11-BODIPY for 30 min at 37 °C. Non-stimulated control cells were treated alongside. All cells were then incubated with 25 μM SUV-7α-OOH for 4 h or 50 μM SUV-7α-OOH for 2.5 h, then washed, overlaid with RPMI medium, and examined by confocal fluorescence microscopy, using a Leica TCS SESII instrument with 488 nm excitation and 607–680 nm (red) or 500–585 nm (green) emission to observe unoxidized and oxidized probe, respectively [19].

For assessing viability, cells were pre-incubated with or without db-cAMP for 3 h, then either left untreated (control) or incubated with liposomal 7α-OOH in increasing concentrations up to 50 μM for an additional 18 h. After incubation, cell viability was assessed by MTT (thiazolyl blue) assay [15].

3. Results

3.1. StarD1, StarD4, and ABCA1 expression in cAMP-stimulated cells: effect of StarD1-kd on ABCA1

We asked first how RAW cell stimulation with db-cAMP would affect expression of StarD1, StarD4, and ABCA1 proteins. StarD1 and StarD4 were detected in resting cells, but ABCA1 was barely detectable (Fig. 1A). StarD1 increased progressively during db-cAMP treatment, reaching about twice its starting level after 9 h. StarD4 was also upregulated, attaining 2.5-times its starting level after 9 h. Non-stimulated cells showed no change in StarD1 or

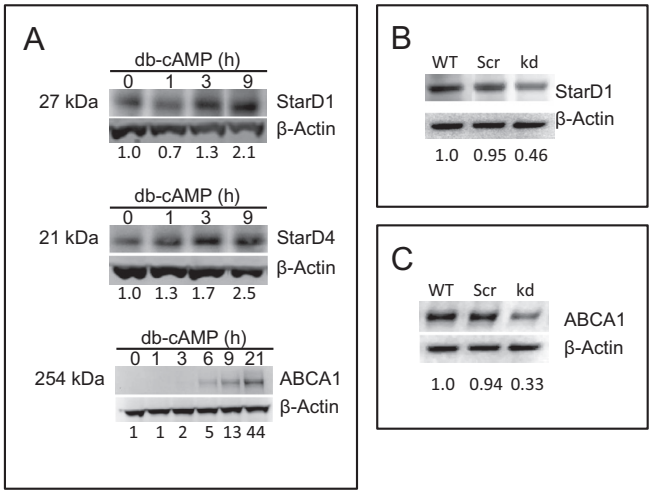

Fig. 1. StarD1, StarD4, and ABCA1 expression in db-cAMP-activated RAW cells: effects of siRNA-based StarD1 knockdown. (A) Cells at 60–70% confluency in serum-free DME medium were incubated with 1 mM db-cAMP for up to 16 h, after which cells were collected, lysed, and subjected to Western analysis for the three proteins along with β-actin as a loading control. Numbers below lanes represent band intensities normalized to β-actin and relative to 0 h. (B) StarD1 and (C) ABCA1 level at 16 h in wild-type, scrambled siRNA-, and siRNA-treated cells. Total protein load: 60 μg/lane.

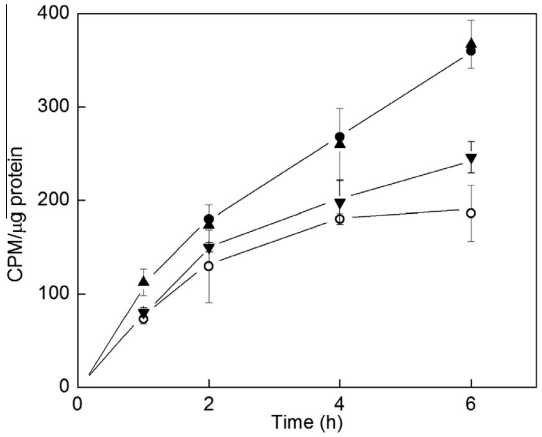

Fig. 2. 7α-OOH uptake by mitochondria in stimulated vs non-stimulated RAW cells; effects of StarD1 knockdown. Wild-type (WT), scrambled siRNA (Scr), and knockdown siRNA (kd) cells stimulated (S) with 1 mM db-cAMP for 9 h in serum-free RPMI, along with a non-stimulated (NS) WT control, were incubated with 50 μM [^{14}C]7α-OOH in POPC/cholesterol/[^{14}C]7α-OOH (1.0:0.8:0.2 by mol) SUVs for the indicated times, after which cells were washed and homogenized. Mitochondria were isolated by differential centrifugation and analyzed by scintillation counting. (●) WT-S; (▲) Scr-S; (▼) kd-S; (○) WT-NS. Means ± S.E. of values from three replicate experiments are plotted.

StarD4 level over these incubation times. There was an especially dramatic upregulation in ABCA1 during db-cAMP treatment, its level after 21 h being more than 40-times above basal (Fig. 1A). StarD1 knockdown (kd) was used to confirm this protein's involvement in RCT [6] and to assess its role in 7α-OOH trafficking. StarD1 level after sequential siRNA and db-cAMP treatment of RAW cells was ~50% that of scrambled control or wild-type cells (Fig. 1B). Significantly, ABCA1 upregulation during cell stimulation was also diminished by StarD1-kd (Fig. 1C), suggesting that StarD1 can regulate ABCA1 expression [6].

3.2. Effect of cell stimulation and StarD1-kd on mitochondrial uptake of 7α-OOH

Knowing that macrophage stimulation with 8-Br-cAMP or db-cAMP enhances cholesterol uptake and export [6,8,9], we asked whether such stimulation would also enhance uptake of 7α-OOH when presented to cells together with cholesterol. As shown in Fig. 2 mitochondria in db-cAMP-stimulated wild-type RAW cells incorporated SUV-supplied [^{14}C]7α-OOH more rapidly than those in non-stimulated controls such that after 6 h, the former contained nearly twice the radioactivity of the latter. Upregulated StarD1 played an important role in the more rapid uptake because StarD1-kd (Fig. 1B) resulted in a substantial slowdown in [^{14}C]7α-OOH uptake, its level at 6 h being only ~67% that of a scrambled siRNA or wild-type control (Fig. 2). Similar effects of StarD1-kd were observed for [^{14}C]cholesterol uptake by stimulated cell mitochondria (not shown), which is consistent with previous findings [6,10]. These results clearly demonstrate that StarD1 can transport not only cholesterol into RAW cell mitochondria, but also 7α-OOH.

3.3. 7α-OOH-induced lipid peroxidation in stimulated vs non-stimulated cells

The membrane-localizing ratiometric fluorophore C11-BODIPY can report on free radical-mediated lipid peroxidation that may occur in its midst. Accordingly, we used this probe to assess whether 7α-OOH internalized by RAW cell mitochondria would initiate lipid peroxidative damage. When exposed to 25 μM SUV-7α-OOH for 4 h, stimulated cells exhibited less red fluorescence (native probe) and more green fluorescence (oxidized probe) than non-stimulated counterparts (Fig. 3A). After 2.5 h with 50 μM SUV-7α-OOH, lipid peroxidation was more advanced overall, but still more intense in stimulated vs non-stimulated cells. Thus, greater 7α-OOH uptake by mitochondria of stimulated cells (Fig. 2) resulted in more extensive peroxidative damage and, as expected, this appeared to be localized mainly in mitochondria (punctate perinuclear zones). Integrated values for the fluorescence signals shown in Fig. 3A are plotted in Fig. 3B as relative (red/green) fluorescence intensities.

3.4. Effect of 7α-OOH on $\Delta\Psi_m$ of stimulated wild-type vs. StarD1-kd cells

Mitochondria damage/dysfunction is commonly reflected in a loss of transmembrane potential ($\Delta\Psi_m$). We used the potentiometric probe JC-1 to assess $\Delta\Psi_m$ status of stimulated vs non-stimulated RAW cells after a 7α-OOH challenge. As shown in Fig. 4A, non-stimulated cells exposed for 5 h to SUV-7α-OOH in increasing concentrations up to 100 μM exhibited an initial (highly reproducible) increase in $\Delta\Psi_m$ at ~20 μM peroxide followed by a rapid decline, reaching complete depolarization at ~50 μM peroxide. In striking contrast, stimulated cells treated identically exhibited an immediate decline in $\Delta\Psi_m$ with increasing 7α-OOH, the nadir in this case occurring at ~30 μM hydroperoxide (Fig. 4A). However, 7α-OH, the redox-inactive reduction product of 7α-OOH, had no effect on $\Delta\Psi_m$ (Fig. 4A) even though it was delivered into stimulated cell mitochondria at the same rate as 7α-OOH (data not shown). Thus, upon arrival in mitochondria (Fig. 2), 7α-OOH underwent free radical turnover, thereby triggering peroxidative damage (Fig. 3) and functional impairment. We established that StarD1 was required for hydroperoxide-induced mitochondria depolarization by showing that time-dependent $\Delta\Psi_m$ loss produced by 100 μM SUV-7α-OOH was substantially slowed by StarD1-kd (Fig. 4B).

3.5. Effect of 7α-OOH on StarD1 and ABCA1 expression in stimulated cells

Knowing that StarD1 and ABCA1 synthesis depends on mitochondria functionality [3,8,9], we asked how mitochondria

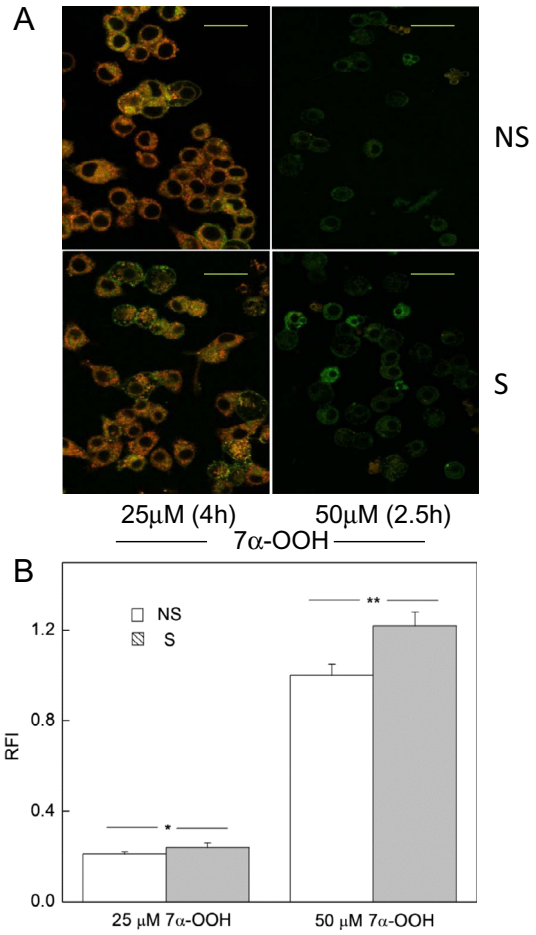

Fig. 3. Lipid peroxidation induced by intracellular 7α-OOH trafficking. (A) Non-stimulated and stimulated cells (1 mM db-cAMP, 9 h) in DMEM medium were incubated with 25 μM 7α-OOH from POPC/cholesterol/7α-OOH (2.5:1.5:1.0 by mol) SUVs for 4 h or 50 μM 7α-OOH from the same SUVs for 2.5 h. Cells were then washed, treated with 2 μM C11-BODIPY for 30 min, and examined by confocal fluorescence microscopy, using settings described in the Section 2. (B) Fluorescence intensity ratio (green/red) plotted as a function of 7α-OOH concentration for cell images shown in (A); at least 100 cells in 5–6 viewing fields are represented in each bar. $^{*}P < 0.05$; $^{**}P < 0.01$.

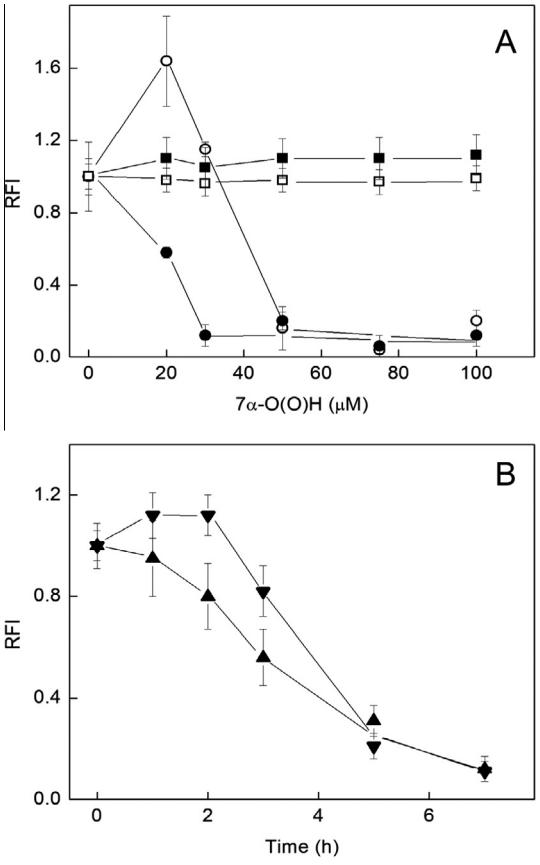

Fig. 4. Effects of 7α-OOH and 7α-OH on $\Delta\Psi_m$ of stimulated vs non-stimulated RAW cells; consequences of StarD1-kd. (A) Wild-type cells in serum-free medium were either not stimulated (NS) or stimulated (S) with 1 mM db-cAMP for 9 h and then incubated with SUV-7α-OOH [(○) NS; (●) S] or SUV-7α-OH [(□) NS; (■) S] in increasing concentrations up to 100 μM for 5 h. (B) Stimulated StarD1-kd (▼) and scrambled siRNA control (▲) cells were incubated with 100 μM SUV-7α-OOH for increasing times up to 7 h. After incubation, cells in (A) and (B) were washed, treated with JC-1 (5 μg/ml) for 30 min, washed again, and examined by fluorescence plate reader, using the following settings: 560 nm ex/595 nm em (red) and 485 nm ex/535 nm em (green). Data are plotted as fluorescence intensity ratios (RFI: 595 nm/535 nm); means ± S.E. of values from 3–4 replicate experiments are represented.

damage/dysfunction caused by 7α-OOH trafficking might affect steady state levels of these proteins. As shown in Fig. 5, exposing 9 h-stimulated RAW cells to 100 μM SUV-7α-OOH resulted in significant decreases in StarD1 and ABCA1 levels (~25% and 50%, respectively), but these were observed after a relatively long incubation time of 6 h. This suggests that loss of antibody-recognizing epitopes was not primarily responsible for these decreases, but rather impaired mitochondrial ATP generation and/or 27-OH production in the case of ABCA1 [8].

3.6. Effect of cell stimulation on 7α-OOH toxicity

In the experiments represented in Figs. 2–5, RAW cells incubated with up to 100 μM SUV-7α-OOH for up to 6 h showed no significant viability losses, but these became more apparent with longer incubation. As shown in Fig. 6, stimulated cells exposed to 10 μM or 50 μM SUV-7α-OOH for 18 h exhibited an MTT-assessed loss of viability that was significantly greater than that of non-stimulated controls. Preliminary experiments revealed that stimulated cell death occurred mainly via intrinsic apoptosis (results not shown), consistent with the mitochondria-targeted oxidative stress represented in Figs. 3 and 4. Thus, 7α-OOH

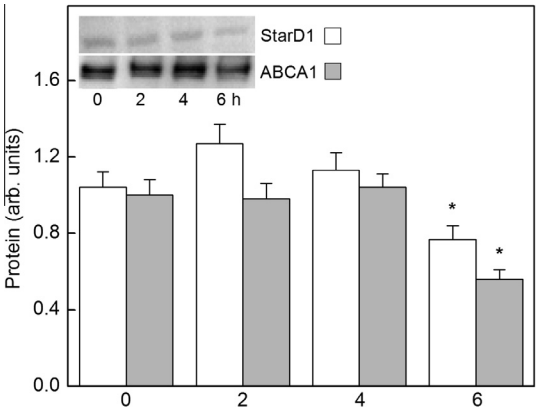

Fig. 5. Effect of 7α-OOH uptake on StarD1 and ABCA1 expression. RAW cells at 60–70% confluency in DME medium were activated for 9 h with 1 mM db-cAMP, then washed and incubated with 100 μM SUV-7α-OOH. At the indicated times, cells were washed, retrieved, lysed and subjected to Western blot analysis for StarD1 and ABCA1. Protein bands were integrated and β-actin-normalized values were plotted relative to starting values (0 h). $^{*}P < 0.01$ compared with 0-time value.

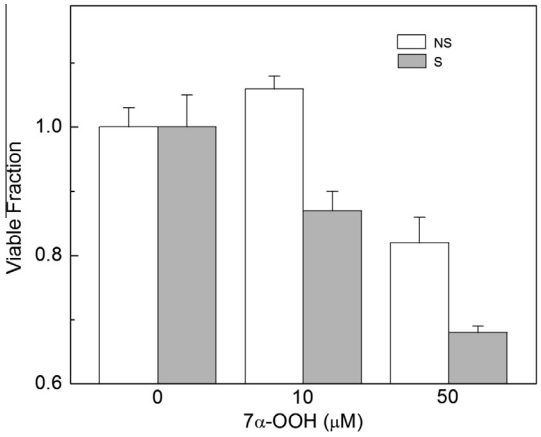

Fig. 6. Effect of cell stimulation on 7α-OOH cytotoxicity. RAW cells in DMEM medium were either not stimulated or stimulated with 1 mM cb-cAMP for 3 h, then challenged with POPC/cholesterol/7α-OOH (1.0:0.8:0.2 by mol) SUVs at the indicated bulk phase concentration of 7α-OOH. After 16 h of incubation, cell viability was assessed by MTT (thiazolyl blue) assay. Means ± S.E. of values from 3–4 separate experiments are plotted relative to non-challenged controls.

produced site-specific redox damage/dysfunction in mitochondria of stimulated RAW cells and this eventually culminated in apoptotic cell death.

4. Discussion

LDL contains numerous unsaturated lipids, including free cholesterol and cholesteryl esters, and is thus susceptible to oxidative modification in the circulation [12,17]. Most prominent among the cholesterol oxidation products are the ChOOHs 7α- and 7β-OOH, the diols 7α- and 7β-OH, the 7-ketone, and 5,6-epoxides [12]. Depending on levels and location, these oxysterols can act as cytotoxic and/or signaling molecules, but only 7α/7β-OOH are redox-active, i.e. capable of inducing peroxidative damage via one-electron reductive turnover [12,13]. In the process, these hydroperoxides are converted to 5,6-epoxides, 7α/7β-OH, and 7-ketone. Modification of LDL apoB-100 protein by 4-hydroxynonenal and other by-products of lipid peroxidation results in a particle (LDL_{ox}) which is more readily taken up by macrophages via scavenger receptors [1]. Uncontrolled uptake and insufficient cholesterol export by macrophages can result in formation of lipid-loaded "foam cells" that accumulate in the vascular wall. This is regarded as a key early event in atherogenesis and it can be exacerbated by oxidative stress associated with disorders such as chronic obesity and hypertension [2].

In this study, we have shown for the first time that 7α-OOH, which has been identified in human LDL_{ox} and atherosclerotic plaques [20,21], can be trafficked to/into mitochondria of db-cAMP-activated RAW264.7 macrophages similarly to cholesterol, thereby causing lipid peroxidative damage, membrane depolarization, metabolic impairment that reduces StarD1 and ABCA1 expression, and ultimately apoptotic cell death. In ongoing studies, similar observations have been made on human THP-1 macrophages (not shown). As observed previously by others [6,10], mitochondrial StarD1 and plasma membrane ABCA1 were strongly upregulated in RAW cells after several hours of db-cAMP treatment. ABCA1 expression was shown to be dependent on StarD1 because knockdown of the latter attenuated the former during cell stimulation. Cytosolic StarD4 was also shown to be upregulated during stimulation, which appears not to have been reported previously. In an earlier study (16), we showed that recombinant StarD4 could bind liposomal 7α-OOH and accelerate its transfer to isolated mitochondria, where peroxide-induced free radical damage/dysfunction ensued. Since a sterol-specific binding pocket exists in most StAR-family proteins (7), it is likely that binding therein was necessary for the 7α-OOH effects observed in the present study. Collectively, these observations support our hypothesis that in vascular macrophages under elevated oxidative pressure, StarD4 and StarD1 will transport ChOOHs from imported LDL_{ox} and/or pre-existing pools to/into mitochondria along with cholesterol, thereby causing internal mitochondria damage. Such damage would disrupt RCT by inhibiting CYP27A1-catalzyed 27-OH generation and thence ABCA1 and ABCG1 induction because 27-OH is a key activator of nuclear LXR, which controls expression of these cholesterol exporters [3–5].

We recently described an analogous mitochondria damage/dysfunction mechanism for steroidogenically-activated MA-10 Leydig cells challenged with 7α-OOH [18]. In that case, we also observed StarD1-dependent transport of 7α-OOH into mitochondria with a concomitant loss of $\Delta\Psi_m$. Moreover, mitochondria-dependent metabolism of cholesterol to progesterone was markedly impaired. Whereas activated MA-10 cells died exclusively by intrinsic apoptosis, non-activated controls died by necrosis, consistent with 7α-OOH delivery to mitochondria in the former case. Preliminary data (not shown) suggested that 7α-OOH-induced death of activated RAW cells (Fig. 6) also occurred via intrinsic apoptosis.

In summary, we have identified a unique mechanism by which stress-generated ChOOHs (represented here by 7α-OOH) can damage and disable macrophages. This introduces a new paradigm of how a particular class of oxysterols, i.e. redox-active ChOOHs that are recognized and transported by StAR family proteins, can incapacitate RCT and thus act to promote atherogenesis [2,22]. One may consider this inauspicious transport to be "stealthy" or "Trojan Horse"-like. A mitochondria-targeted antioxidant such as mitochondria-Q, which is being clinically evaluated for other purposes [23], may prove useful for suppressing the damage/dysfunction we describe, but this remains to be investigated.

Acknowledgments

This work was supported by Polish National Science Center Grant 2011/01/B/NZ3/02167 and Medical College of Wisconsin Research Affairs Committee Grant 3726 (to W.K.) and by National Institutes of Health Grant HL85677 (to A.W.G). We thank Dr. Jerzy Dobrucki for making the confocal microscope available in the Laboratory of Cell Biophysics.

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