Biochemical and Biophysical Research Communications 446 (2014) 714–719

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Induction of oxiapoptophagy, a mixed mode of cell death associated with oxidative stress, apoptosis and autophagy, on 7-ketocholesterol-treated 158N murine oligodendrocytes: Impairment by α-tocopherol

Thomas Nury [a], Amira Zarrouk [a,b], Anne Vejux [a], Margaux Doria [a], Jean Marc Riedinger [c], Régis Delage-Mourroux [d], Gérard Lizard [a,*]

[a] Equipe 'Biochimie du Peroxysome, Inflammation et Métabolisme Lipidique' EA 7270/Université de Bourgogne/INSERM, Dijon, France
[b] Université de Monastir, Faculté de Médecine, LR12ES05-Lab-NAFS, Monastir, Tunisia
[c] Centre de Lutte Contre le Cancer GF Leclerc, Laboratoire de Biologie Médicale, Dijon, France
[d] UFR Sciences et Techniques EA3922/SFR IBCT FED 4234, Université de Franche-Comté, Besançon, France

ARTICLE INFO

Article history:
Available online 2 December 2013

Keywords:
7-Ketocholesterol
α-Tocopherol
158N cells
Apoptosis
Autophagy
Oxiapoptophagy
Oxidative stress

ABSTRACT

7-Ketocholesterol (7KC) has been suggested to induce a complex mode of cell death on monocytic cells: oxiapoptophagy (OXIdation, APOPTOsis, and autoPHAGY) (Monier et al. (2003) [12]). The aim of the present study, realized on 158N murine oligodendrocytes, was to bring new evidence on this mixed form of cell death. On 158N cells, 7KC induces an overproduction of reactive oxygen species (ROS) revealed by dihydroethidium staining, a loss of transmembrane mitochondrial potential measured with $DiOC_6(3)$, caspase-3 activation, and condensation and/or fragmentation of the nuclei which are typical criteria of oxidative stress and apoptosis. Moreover, 7KC enhances cytoplamic membrane permeability to propidium iodide, and induces acidic vesicular organelle formation evaluated with acridine orange. In addition, 7KC promotes conversion of microtubule-associated protein light chain 3 (LC3-I) to LC3-II which is characteristic of autophagy. These different side effects were impaired by α-tocopherol. Altogether, our data demonstrate that oxiapoptophagy including ROS overproduction, apoptosis and autophagy could be a particular type of cell death activated by 7KC which can be inhibited by α-tocopherol.

1. Introduction

Cholesterol oxide products (oxysterols) are oxidized forms of cholesterol or its precursors. These compounds, which can be obtained under the action of endogenous enzymes (cytochrome P450 enzymes, hydroxylases, dehydrogenases) or by autoxidation, are present in various food constituents and also formed endogeneously [1,2]. A number of these products are biologically active, and can have various side effects, especially those oxidized at C7 such as 7-ketocholesterol (7KC). This oxysterol is known to induce oxidative stress, inflammatory processes and cell death mainly on monocytic cells, and it is suspected to contribute to the development of various age-related diseases including cardiovascular diseases, age-related macular degeneration, and neurodegenerative diseases [3,4]. In atherosclerosis, 7KC is present at enhanced levels in oxidized-low density lipoproteins (Ox-LDL), which accumulate in the intima of atherosclerotic patients; it is known to mediate various side effects of Ox-LDL leading to foam cells formation and to the development of atherosclerotic plaques [5,6]. In age-related macular degeneration, the potential implication of oxysterols, especially those generated by non enzymatic reactions as 7KC, is strongly suspected [7]. The level of 7KC is also twice higher in Parkinson's disease brains versus control brains [8]. In multiple sclerosis, significantly higher levels of 7KC were also found in the cerebrospinal fluid (CSF) of multiple sclerotic patients when compared with the CSF of patients with non-inflammatory neurological diseases [9]. In X-linked adrenoleukodystrophy, which is the most frequent peroxisomal leukodystrophy, the early activation of oxidative stress may also contribute to oxidizing cholesterol, leading to the formation of oxysterols oxidized at C7 such as 7KC [10]. Therefore, it is likely that 7KC, via its different side effects, might contribute to demyelinization by its ability to trigger cell death on oligodendrocytes. Currently, the capacity of 7KC to induce apoptosis on oligodendrocytes is well established, and the simultaneous involvement of oxidative stress is supported by the aptitude of α- to counteract 7KC-induced cell death [11]. In addition, the involvement of autophagic processes in 7KC-induced

Abbreviations: 7KC, 7-ketocholesterol; AO, acridine orange; CSF, cerebrospinal fluid; DHE, dihydroethidium; LC3-I, microtubule-associated protein light chain 3-I; LC3-II, microtubule-associated protein light chain 3-II; Ox-LDL, oxidized-low density lipoproteins; ROS, reactive oxygen species.

* Corresponding author. Address: Laboratoire BIO-peroxIL ('Biochimie du Peroxysome, Inflammation et Métabolisme Lipidique') – EA 7270/INSERM, Faculté des Sciences Gabriel, 6 Bd Gabriel, 21000 Dijon, France. Fax: +33 380 39 62 50.
E-mail address: gerard.lizard@u-bourgogne.fr (G. Lizard).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.11.081

cell death is widely suspected on human monocytic U937 cells, and supports the hypothesis that 7KC might induce a complex mode of cell death on these cells: oxiapoptophagy (OXIdation, APOPTOsis, and autoPHAGY) [12,13]. Currently, abnormal autophagic activities are frequently observed in common neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis) [14]. Therefore, metabolic pathways associated with autophagy can constitute potential new therapeutic targets for common neurodegenerative diseases. So, it was of interest to determine on oligodendrocytes, which are myelin synthetizing cells in the central nervous system, whether 7KC-induced oxidative stress and apoptosis can be simultaneously associated with autophagy.

To this end, 158N murine oligodendrocytes were cultured in the absence or presence of 7KC associated or not with α-tocopherol known to counteract 7KC-induced apoptosis on these cells [11]. Our data demonstrate that 7KC induces a mixed type of cell death on 158N cells, including reactive oxygen species (ROS) overproduction, apoptosis and autophagy, which can be considered as oxiapoptophagy, and which is impaired by simultaneous treatment with α-tocopherol.

2. Materials and methods

2.1. Cell culture and treatments

Normal murine oligodendrocytes (158N) [15] were seeded at 5000–10,000 cells/cm^2 either in Petri dishes (100 mm in diameter), or six-well plates, and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum and 1% antibiotics (penicillin, streptomycin). The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2, trypsinized (0.05% trypsin-0.02% EDTA solution), and passaged twice a week.

Initial solutions of 7KC (Sigma–Aldrich, St. Quentin Fallavier, France) were prepared at 800 μg/mL [11]. 7KC concentrations were chosen in the range of those inducing cell death on numerous cell types [3]. After 24 h of culture, subconfluent 158N cells were incubated with 7KC (5, 10, 20, and/or 40 μg/mL) corresponding to (12.5, 25, 50, and 100 μM) for 6, 14, 18, and/or 24 h. When 158N cells were simultaneously treated with 7KC and α-tocopherol (400 μM) (Sigma–Aldrich), α-tocopherol was added 2 h before 7KC [11].

2.2. Analysis of cell morphology by phase contrast microscopy

Cell morphology was observed after 24 h of treatment in the absence or presence of 7KC (5, 10, 20, and/or 40 μg/mL) associated or not with α-tocopherol (400 μM) under an inverted-phase contrast microscope (Axiovert 40CFL, Zeiss, Jena, Germany). Digitized images were obtained with a camera (Axiocam ICm1, Zeiss).

2.3. Staining with crystal violet

Quantification of adherent cells was estimated by staining with crystal violet [11]. Cells were seeded in triplicates in 6 well plates, and cultured without or with 7KC (5, 10, 20, and/or 40 μg/mL) associated or not with α-tocopherol (400 μM) for 24 h. At the end of treatment, cells were washed with PBS, stained with crystal violet (Sigma–Aldrich) (5 min), and rinsed with water. Absorbance was read at 570 nm after extraction of the dye with 0.1 mol/L sodium citrate in 50% ethanol.

2.4. Evaluation of oxidative stress with dihydroethydium

Overproduction of ROS, mainly superoxide anion (O_2^-), was detected with dihydroethidium (DHE; Life Technologies, Life Technologies, St. Aubin, France) [16]. DHE (1.6 mM) was prepared in dimethyl sulfoxide and used at 2 μM. The fluorescent signals were collected through a 590/20 nm band pass filter on a logarithmic scale on a GALAXY flow cytometer (Partec, Münster, Germany); 10,000 cells were acquired; data were analyzed with Flomax (Partec) or FlowJo (Tree Star Inc., Ashland, USA) softwares.

2.5. Measurement of transmembrane mitochondrial potential with $DiOC_6(3)$

Variations of the mitochondrial transmembrane potential ($\Delta\Psi_m$) were measured with 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6(3)$; Life Technologies). Adherent and non-adherent cells were pooled, and stained with $DiOC_6(3)$ (40 nM) [11]. Mitochondrial depolarization is indicated by a decrease in green fluorescence collected through a 520/10 nm band pass filter on a Galaxy flow cytometer (Partec). Ten thousand cells were acquired for each sample. Data were analyzed with Flomax (Partec) or FlowJo (Tree Star Inc.) softwares.

2.6. Quantification of acidic vesicular organelle formation with Acridine orange

Acridine orange (AO) is a weak base which accumulates in its charged form within lysosomes of living cells because of the low lysosomal pH, and which produces a red fluorescence when excited by a blue light [17]. AO is widely used to visualize and quantify autophagic vesicles [18]. A 1 mg/mL stock solution of AO (Sigma–Aldrich) was prepared in distilled water. After staining with AO (2 μg/mL; 15 min; 37 °C), cells were washed, resuspended in PBS, and analyzed by flow cytometry. The fluorescent signals were measured on a Galaxy flow cytometer (Partec). The orange fluorescence of AO was collected through a 590/20 nm band pass filter. Fluorescence was quantified on 10,000 cells. Data were analyzed with Flomax (Partec) or FlowJo (Tree Star Inc.) softwares.

2.7. Quantification of dead cells by staining with propidium iodide

Adherent and non-adherent cells were pooled and stained with propidium iodide (PI (Sigma–Aldrich); 1 μg/mL, 5 min), which enters dead cells only [19]. The cells were analyzed with a Galaxy flow cytometer (Partec). Red fluorescence of PI was detected through a 630 nm long pass filter. For each sample, 10,000 cells were acquired. Data were analyzed with the FlowMax (Partec) or FlowJo (Tree Star Inc.) softwares.

2.8. Nuclei staining with Hoechst 33342

Nuclear morphology of cells cultured without or with 7KC (20 μg/mL) associated or not with α-tocopherol (400 μM) was characterized by fluorescence microscopy after staining with Hoechst 33342 (Sigma–Aldrich; 2 μg/mL) [11,19]. Briefly, cell deposits were applied to glass slides by cytocentrifugation, mounted in Dako fluorescent mounting medium (Dako, Copenhagen, Denmark) and the morphological aspect of the cell nuclei was determined with an Axioskop fluorescent microscope (Zeiss). For each sample, 300 cells were examined.

2.9. Protein analysis by polyacrylamide gel electrophoresis and Western blotting

Cells were lysed in a Ripa buffer (10 mM Tris–HCl, pH 7.2, 150 mM NaCl, 0.5% Nonidet NP40, 0.5% Na deoxycholate, 0.1% SDS, 2 mM EDTA and 50 mM NaF) in the presence of 1/25 complete protease inhibitor cocktail tablets (Roche Diagnostics Corporation, Indianapolis, IN, USA) for 30 min on ice. Cell lysates were cleared

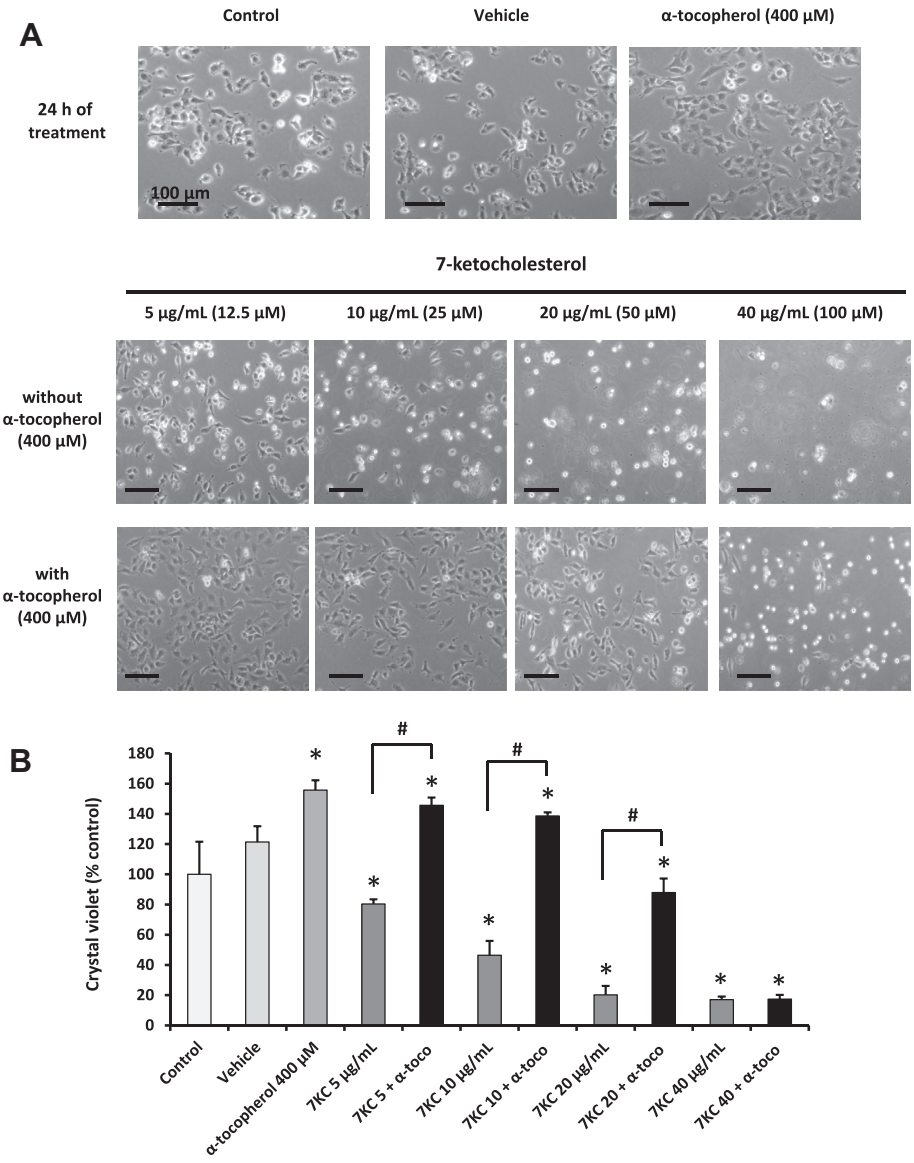

Fig. 1. Dose-dependent effects of 7-ketocholesterol associated or not with α-tocopherol on cell growth. 158N murine oligodendrocytes were cultured for 24 h in the absence or presence of 7KC (5, 10, 20, and 40 μg/mL) without or with α-tocopherol (400 μM). The effect on cells growth was evaluated by phase contrast microscopy (A) and by staining with crystal violet (B) to quantify adherent cells. Vehicle (Ethanol: 0.1%) corresponds to the highest ethanol concentration used to dissolve 7KC. Data are mean ± SD from two independent experiments conducted in triplicates. Significance of the difference between vehicle- and 7KC-treated cells; Mann Whitney test: $^{*}P < 0.05$. With crystal violet test, no significant difference was observed between control and vehicle-treated cells. Significance of the difference between 7KC-treated cells and (α-tocopherol + 7KC)-treated cells; Mann Whitney test: $^{\#}P < 0.05$.

by a 15 min centrifugation at 20,000*g*. The protein concentration was measured in the supernatant using the Bicinchoninic Acid Solution (Sigma–Aldrich). Fifty to eighty micrograms of proteins were diluted in loading buffer (125 mM Tris–HCl, pH 6.8, 10% β-mercaptoethanol, 4.6% SDS, 20% glycerol, and 0.003% bromophenol blue), separated on a polyacrylamide SDS-containing gel, and transferred onto a nitrocellulose membrane (Thermo-Scientific, Waltham, MA, USA). After blocking nonspecific binding sites for 1 h with 5% nonfat milk in TBST (10 mM Tris–HCl, 150 mM NaCl, 0.1% Tween 20, pH 8), the membrane was incubated overnight with the primary antibody diluted in TBST with 1–5% milk. The antibodies raised against active caspase-3 (#9662) and LC3-I/II (L8918) were from Cell Signaling (Ozyme, Saint Quentin Yvelines, France), and (Sigma–Aldrich), and used at 1/1000 final concentration; respectively. Antibody directed against β-actin (Sigma–Aldrich) was used at 1/10,000 final concentration. The membrane was then washed with TBST and incubated (1 h, room temperature) with horseradish peroxidase-conjugated goat anti-mouse (Santa-Cruz Biotechnology) or anti-rabbit antibody (Santa-Cruz Biotechnology or Cell Signaling) diluted at 1/5000. The membrane was washed with TBST and revealed using an enhanced chemiluminescence detection kit (Supersignal West Femto Maximum Sensitivity Substrate, Thermo-Scientific) and Chemidoc XRS+(Bio-Rad). The level of cleaved caspase-3 was determined versus actin, and the ratio LC3-II/LC3-I was calculated with Image Lab software (Bio-Rad).

2.10. Statistical analysis

Analyses were carried out with WinSTAT® for Microsoft® Excel (version 2012.1) with the Mann–Whitney test. Data were considered statistically different at a *P*-value of 0.05 or less.

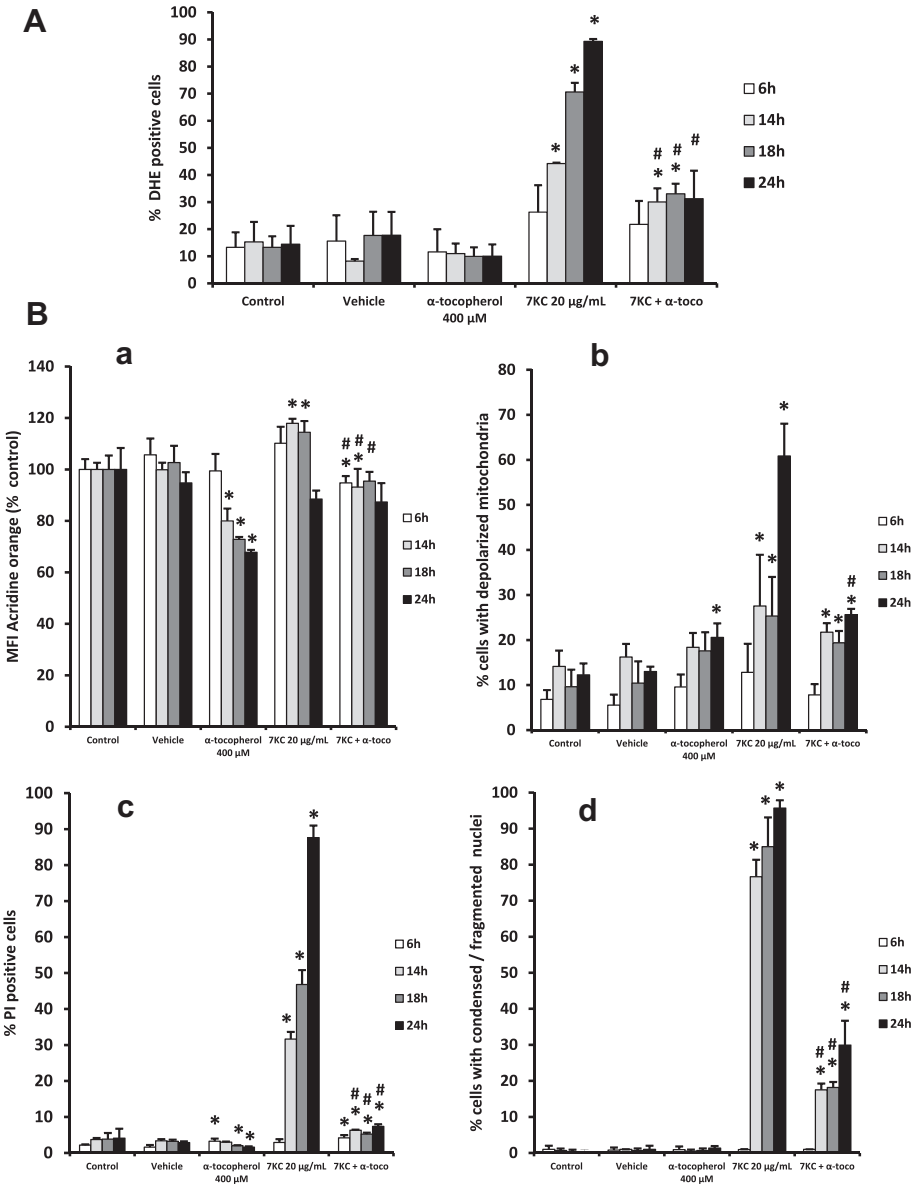

Fig. 2. Time-dependent effects of 7-ketocholesterol associated or not with α-tocopherol on oxidative stress, acidic organelle formation, and cell death induction. 158N murine oligodendrocytes were cultured for 24 h in the absence or presence of 7KC (20 µg/mL) without or with α-tocopherol (400 µM). The percentage of cells overproducing ROS was determined with DHE (A), the impact on various organelles and cell death was determined with different methods (B): (a) formation of acidic organelle (including autophagic vesicles) with AO; (b) mitochondrial depolarization with $DiOC_6(3)$; (c) cytoplasmic membranes damages (allowing quantification of dead cells) with PI; (d) quantification of cells with condensed and/or fragmented nuclei (characteristic of apoptotic cells) with Hoechst 33342. Vehicle (Ethanol: 0.1%) corresponds to the highest ethanol concentration used to dissolve 7KC. Significance of the difference between vehicle- and 7KC-treated cells; Mann Whitney test: $^{*}P < 0.05$. No significant difference was observed between control and vehicle-treated cells. Significance of the difference between 7KC-treated cells and (α-tocopherol + 7KC)-treated cells; Mann Whitney test: $^{\#}P < 0.05$.

3. Results

3.1. Evaluation of the effects of 7-ketocholesterol associated or not with α-tocopherol on 158N cells by phase contrast microscopy and crystal violet test

Under treatment of 158N cells with 7KC (5, 10, 20 and 40 µg/mL) for 24 h, marked dose dependent effects on cell adhesion and cell growth were observed. At 10, 20, and 40 µg/mL, reduced numbers of adherent cells were revealed by phase contrast microscopy and crystal violet test comparatively to untreated cells, vehicle (ethanol 0.1%)-, and α-tocopherol (400 µM)-treated cells (Fig. 1A and B). Simultaneously, increases of cells floating in the cultured medium were detected (Fig. 1A). With 7KC (10–20 µg/mL), these side effects were counteracted by α-tocopherol (400 µM). However, no protective effects of α-tocopherol were observed with 7KC (40 µg/mL) (Fig. 1A and B). Therefore, for further experiments, 7KC was used at 20 µg/mL (the highest concentration inducing side effects), which is impaired by α-tocopherol.

3.2. Evaluation of the effects of 7-ketocholesterol associated or not with α-tocopherol on oxidative stress, acidic organelle formation, and cell death induction

Under treatment of 158N cells with 7KC (20 µg/mL; 6, 14, 18 and 24 h), marked time dependent increase on ROS overproduction was observed comparatively to untreated cells, vehicle (ethanol 0.1%)-, and α-tocopherol (400 µM)-treated cells (Fig. 2A). Increases of acidic organelles stained with AO (revealed by increased Mean Fluorescence Intensity (MFI) values), which can correspond to

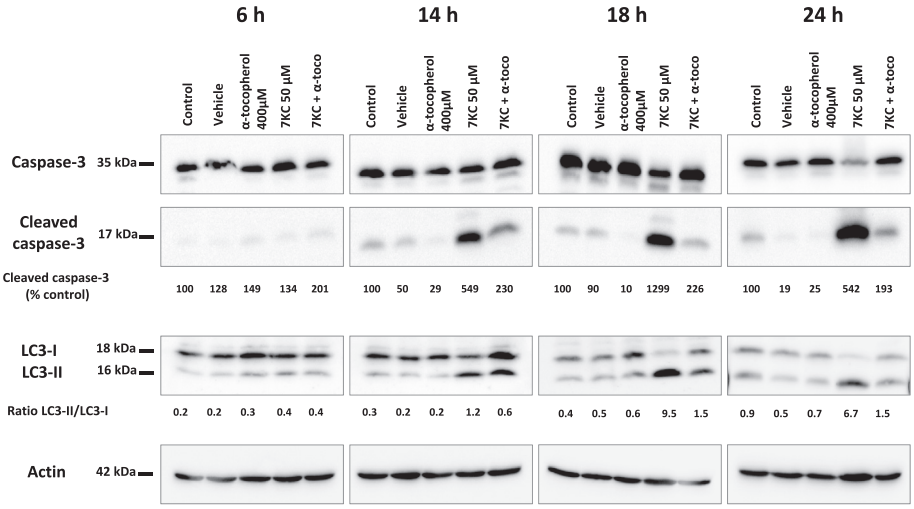

Fig. 3. Time-dependent effects of 7-ketocholesterol associated or not with α-tocopherol on apoptosis, and on autophagy. 158N murine oligodendrocytes were cultured for 24 h in the absence or presence of 7KC (20 μg/mL) without or with α-tocopherol (400 μM). Apoptosis and autophagy were characterized by Western blotting with different antibodies. Apoptosis was evaluated by caspase-3 activation (cleaved caspase-3; % control) and autophagy by conversion of LC3-I to LC3-II [ratio LC3-II/LC3-I]. No difference was observed between control and vehicle-treated cells.

autophagic vesicles, were also observed with 7KC mainly at 6, 14, and 18 h (Fig. 2B); the decrease of MFI observed at 24 h (Fig. 2B) suggest a loss of lysosomal membrane integrity previously reported on U937 cells treated with 7KC for long periods of time [20]. Moreover, 7KC-induced apoptosis revealed by high percentages of apoptotic cells (70–98%) at 14, 18, and 24 h was associated with marked increased of cells with depolarized mitochondria and of cells with damaged cytoplasmic membranes revealed by staining with DiOC6(3) and PI, respectively (Fig. 2B). The increases of ROS overproduction, acidic vesicles formation, cells with depolarized mitochondria, and cells with condensed and/or fragmented nuclei (apoptotic cells) were reduced by α-tocopherol at 14, 18, and/or 24 h (Fig. 2A and B). These data demonstrate that 7KC-induced apoptosis is associated with oxidative stress, acidic vacuoles formation, and that α-tocopherol impairs these side effects.

3.3. Evaluation of the effects of 7-ketocholesterol associated or not with α-tocopherol on apoptosis, and autophagy

To demonstrate the occurrence of apoptosis and autophagy under treatment with 7KC (20 μg/mL; 6, 14, 18 and 24 h), the presence of cleaved caspase-3 and the conversion of microtubule-associated protein light chain 3 (LC3-I) to LC3-II (evaluated by the ratio LC3-II/LC3-I) was studied by Western blotting (Fig. 3). In these conditions, cleaved caspase-3 was detected at 14, 18, and 24 h. As an increased of the ratio [LC3-II/LC3-I] was observed at the same times, this demonstrates the simultaneous occurrence of apoptosis and autophagy under treatment with 7KC. The induction of cleaved caspase-3 and the conversion of LC3-I to LC3-II were strongly reduced by α-tocopherol (Fig. 3). These data demonstrate that 7KC simultaneously induces apoptosis and autophagy which are impaired by α-tocopherol.

4. Discussion

The ability of 7KC to induce apoptosis on numerous cell types is now well established [3], and some investigations realized on monocytic and smooth muscles cells [12,13,21–23] and on tissue sections from atherosclerotic lesions [22,24] support that this oxysterol could induce autophagy *in vitro* and *in vivo*. As 7KC induces numerous side effects (oxidative stress, inflammatory processes, and cell death) [20], the term oxiapoptophagy [12] was suggested to define the complex mode of cell death induced by 7KC on U937 cells including oxidative stress, apoptosis, and probably also autophagy [13]. As there are some evidences of autophagy in numerous brain diseases [14] associated with increased levels of 7KC [8–10], it was of interest to determine (i) whether autophagy could be induced by 7KC on oligodendrocytes simultaneously with oxidative stress and apoptosis, and (ii) whether autophagy could be inhibited by α-tocopherol which is known to counteract 7KC-induced apoptosis [11]. Our data demonstrate that 7KC-induced oxidative stress is associated with a simultaneous induction of apoptosis and autophagy, and that α-tocopherol impairs these different side effects.

Under treatment with 7KC, the overproduction of ROS (especially O_2^-) observed on 158N murine oligodendrocytes underlines that this oxysterol is not only able to trigger oxidative stress on monocytic and vascular cells [3] but also on brain cells. As an involvement of various NADPH isoforms leading to O_2^- overproduction has been shown in several cell types treated with 7KC [3,22], it would be interesting to define on oligodendrocytes the part taken by this enzyme, which constitutes a potential pharmacological target.

Moreover, in agreement with data obtained on J-774 murine macrophages treated with a cholesterol oxide mixture containing 7KC [25], our data show that 7KC induces at early time acidic organelle modifications wich could correspond to autophagosome formation [18] previously suggested by the presence of numerous monodansylcadaverine positive structures in the cytoplasm of 7KC-treated monocytic U937 cells [26]. At prolonged time of treatment, 7KC induces a lysosomal destabilization which might lead to the release of lysosomal enzymes into the cytosol and which could be critical for cell death induction [20,25].

In addition, as previously reported on 158N murine oligodendrocytes, 7KC triggers an apoptotic mode of cell death associated with a loss of transmembrane mitochondrial potential involving caspase-3 activation [11], and with an increased permeability to PI, which could be at least in part a consequence of lipid membrane peroxidation. In this complex mode of cell death, oxidative stress is probably a key event as suggested by the ability of α-tocopherol to prevent 7KC-induced apoptosis. This hypothesis is supported by data obtained on different cell types with various anti-oxidants (N-acetyl cysteine, reduced glutathione) and with α-tocopherol

which have the ability to counteract 7KC-induced mitochondrial depolarization and the cascade of events leading to the final step of apoptosis characterized by the occurrence of cells with fragmented nuclei [3].

Moreover, as 7KC also regulates the expression of autophagic genes [24], and has been shown to trigger autophagy on vascular smooth muscle cells and coronary arterial myocytes [21,23], we asked whether 7KC could simultaneously induce apoptosis and autophagy on 158N murine oligodendrocytes. The ability of 7KC to simultaneously trigger apoptosis and autophagy is based on the hypothesis proposed on 7KC-treated U937 cells [12], and is supported by studies which revealed interdependence between the pathways leading to major forms of cell death such as autophagy, apoptosis, mitoptosis, necrosis, and necroptosis [27,28]. In the present study, the concomitent induction of autophagy and apoptosis by 7KC was shown by western blotting by the conversion of microtubule-associated protein light chain 3 (LC3-I) to LC3-II, which is a specific marker of autophagy, and by the simultaneous induction of cleaved caspase-3.

Therefore, our data demonstrate that 7KC induces a mixed type of cell death on 158N murine oligodendrocytes simultaneously involving oxidative stress, apoptosis, and autophagy which could be defined as oxiapoptophagy. They also highlight the α-tocopherol ability to prevent 7KC-induced oxiapoptophagy: ROS overproduction, cascade of apoptosis associated events, and conversion of LC3-I to LC3-II. It remains to define how these different events are interconnected, and whether autophagy contributes to down- or up-regulate 7KC-induced cell death. So, design of appropriated molecules is of interest to prevent various cardiovascular and neurodegenerative diseases associated with elevated 7KC levels.

Acknowledgments

This work was supported by grants from the Université de Bourgogne and the Conseil Régional de Bourgogne; it was presented as oral presentation at the 3rd ENOR (European Network on Oxysterol Research) symposium 'OXYSTEROLS: markers and Pathways' in Swansea, UK, 19-20 september 2013.

References

[1] W.J. Griffiths, P.J. Crick, Y. Wang, Methods for oxysterol analysis: past, present and future, Biochem. Pharmacol. 86 (2013) 3–14.
[2] L. Iuliano, Pathways of cholesterol oxidation via non-enzymatic mechanisms, Chem. Phys. Lipids 164 (2011) 457–468.
[3] A. Vejux, G. Lizard, Cytotoxic effects of oxysterols associated with human diseases: induction of cell death (apoptosis and/or oncosis), oxidative and inflammatory activities, and phospholipidosis, Mol. Aspects Med. 30 (2009) 153–170.
[4] G. Poli, F. Biasi, G. Leonarduzzi, Oxysterols in the pathogenesis of major chronic diseases, Redox Biol. 1 (2013) 125–130.
[5] Q. Zhou, E. Wasowicz, B. Handler, B. Handler, L. Fleischer, F.A. Kummerow, An excess concentration of oxysterols in the plasma is cytotoxic to cultured endothelial cells, Atherosclerosis 149 (2000) 191–197.
[6] S.M. Colles, J.M. Maxson, S.G. Carlson, G.M. Chisolm, Oxidized LDL-induced injury and apoptosis in atherosclerosis. Potential roles for oxysterols, Trends Cardiovasc. Med. 11 (2001) 131–138.
[7] I.R. Rodríguez, I.M. Larrayoz, Cholesterol oxidation in the retina: implications of 7KCh formation in chronic inflammation and age-related macular degeneration, J. Lipid Res. 51 (2010) 2847–2862.
[8] D. Cheng, A.M. Jenner, G. Shui, W.F. Cheong, T.W. Mitchell, J.R. Nealon, W.S. Kim, H. McCann, M.R. Wenk, G.M. Halliday, B. Garner, Lipid pathway alterations in Parkinson's disease primary visual cortex, PLoS One 6 (2) (2011) e17299.
[9] V. Leoni, T. Masterman, F.S. Mousavi, B. Wretlind, L.O. Wahlund, U. Diczfalusy, J. Hillert, I. Björkhem, Diagnostic use of cerebral and extracerebral oxysterols, Clin. Chem. Lab. Med. 42 (2004) 186–191.
[10] M. Baarine, P. Andréoletti, A. Athias, T. Nury, A. Zarrouk, K. Ragot, A. Vejux, J.M. Riedinger, Z. Kattan, G. Bessede, D. Trompier, S. Savary, M. Cherkaoui-Malki, G. Lizard, Evidence of oxidative stress in very long chain fatty acid-treated oligodendrocytes and potentialization of ROS production using RNA interference-directed knockdown of ABCD1 and ACOX1 peroxisomal proteins, Neuroscience 213 (2012) 1–18.
[11] K. Ragot, J.J. Mackrill, A. Zarrouk, T. Nury, V. Aires, A. Jacquin, A. Athias, J.P. Pais de Barros, A. Véjux, J.M. Riedinger, D. Delmas, G. Lizard, Absence of correlation between oxysterol accumulation in lipid raft microdomains, calcium increase, and apoptosis induction on 158N murine oligodendrocytes, Biochem. Pharmacol. 86 (2013) 67–79.
[12] S. Monier, M. Samadi, C. Prunet, M. Denance, A. Laubriet, A. Athias, A. Berthier, E. Steinmetz, G. Jürgens, A. Nègre-Salvayre, G. Bessède, S. Lemaire-Ewing, D. Néel, P. Gambert, G. Lizard, Impairment of the cytotoxic and oxidative activities of 7 beta-hydroxycholesterol and 7-ketocholesterol by esterification with oleate, Biochem. Biophys. Res. Commun. 303 (2003) 814–824.
[13] A. Vejux, S. Guyot, T. Montange, J.M. Riedinger, E. Kahn, G. Lizard, Phospholipidosis and down-regulation of the PI3-K/PDK-1/Akt signalling pathway are vitamin E inhibitable events associated with 7-ketocholesterol-induced apoptosis, J. Nutr. Biochem. 20 (2009) 45–61.
[14] J.H. Son, J.H. Shim, K.H. Kim, J.Y. Ha, J.Y. Han, Neuronal autophagy and neurodegenerative diseases, Exp. Mol. Med. 44 (2012) 89–98.
[15] M. Baarine, K. Ragot, E.C. Genin, H. El Hajj, D. Trompier, P. Andreoletti, M.S. Ghandour, F. Menetrier, M. Cherkaoui-Malki, S. Savary, G. Lizard, Peroxisomal and mitochondrial status of two murine oligodendrocytic cell lines (158N, 158JP): potential models for the study of peroxisomal disorders associated with dysmyelination processes, J. Neurochem. 111 (2009) 119–131.
[16] A. Zarrouk, A. Vejux, T. Nury, H.I. El Hajj, M. Haddad, M. Cherkaoui-Malki, J.M. Riedinger, M. Hammami, G. Lizard, Induction of mitochondrial changes associated with oxidative stress on very long chain fatty acids (C22:0, C24:0, or C26:0)-treated human neuronal cells (SK-NB-E), Oxid. Med. Cell. Longev. 2012 (2012) 623257.
[17] M. Olsson, I. Rundquist, U. Brunk, Flow cytofluorometry of lysosomal acridine orange uptake by living cultured cells, Acta Path. Microbiol. Immunol. Scand. (Sect A) 95 (1987) 159–165.
[18] A. Altmeyer, A.C. Jung, M. Ignat, S. Benzina, J.M. Denis, J. Gueulette, G. Noël, D. Mutter, P. Bischoff, Pharmacological enhancement of autophagy induced in a hepatocellular carcinoma cell line by high-LET radiation, Anticancer Res. 30 (2010) 303–310.
[19] G. Lizard, S. Fournel, L. Genestier, N. Dhedin, C. Chaput, M. Flacher, M. Mutin, G. Panaye, J.P. Revillard, Kinetics of plasma membrane and mitochondrial alterations in cells undergoing apoptosis, Cytometry 21 (1995) 275–283.
[20] S. Lemaire-Ewing, C. Prunet, T. Montange, A. Vejux, A. Berthier, G. Bessède, L. Corcos, P. Gambert, D. Néel, G. Lizard, Comparison of the cytotoxic, pro-oxidant and pro-inflammatory characteristics of different oxysterols, Cell. Biol. Toxicol. 21 (2005) 97–114.
[21] W. Martinet, D.M. Schrijvers, J.P. Timmermans, H. Bult, Interactions between cell death induced by statins and 7-ketocholesterol in rabbit aorta smooth muscle cells, Br. J. Pharmacol. 154 (2008) 1236–1246.
[22] C. He, H. Zhu, W. Zhang, I. Okon, Q. Wang, H. Li, Y.Z. Le, Z. Xie, 7-Ketocholesterol induces autophagy in vascular smooth muscle cells through Nox4 and Atg4B, Am. J. Pathol. 183 (2013) 626–637.
[23] M. Xu, X.X. Li, J. Xiong, M. Xia, E. Gulbins, Y. Zhang, P.L. Li, Regulation of autophagic Flux by dynein-mediated autophagosomes trafficking in mouse coronary arterial myocytes, Biochim. Biophys. Acta (2013). pii: S0167-4889(13)00337-6.
[24] G. Jia, G. Cheng, D.K. Agrawal, Autophagy of vascular smooth muscle cells in atherosclerotic lesions, Autophagy 3 (2007) 63–64.
[25] X.M. Yuan, W. Li, U.T. Brunk, H. Dalen, Y.H. Chang, A. Sevanian, Lysosomal destabilization during macrophage damage induced by cholesterol oxidation products, Free Radic. Biol. Med. 28 (2000) 208–218.
[26] C. Miguet-Alfonsi, C. Prunet, S. Monier, G. Bessède, S. Lemaire-Ewing, A. Berthier, F. Ménétrier, D. Néel, P. Gambert, G. Lizard, Analysis of oxidative processes and of myelin figures formation before and after the loss of mitochondrial transmembrane potential during 7beta-hydroxycholesterol and 7-ketocholesterol-induced apoptosis: comparison with various pro-apoptotic chemicals, Biochem. Pharmacol. 64 (2002) 527–541.
[27] L. Galluzzi, G. Kroemer, Necroptosis: a specialized pathway of programmed necrosis, Cell 135 (2008) 1161–1163.
[28] W. Chaabane, S.D. User, M. El-Gazzah, R. Jaksik, E. Sajjadi, J. Rzeszowska-Wolny, M.J. Los, Autophagy, apoptosis, mitoptosis and necrosis: interdependence between those pathways and effects on cancer, Arch. Immunol. Ther. Exp. (Warsz) 61 (2013) 43–58.