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Oligomerized Amyloid-$\beta_{1\text{-}40}$ Peptide Favors Cholesterol, Oxysterol, and Fatty Acid Accumulation in Human Neuronal SK-N-BE Cells

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Abstract

Amyloid peptide, the main component of senile plaques, is a major biological characteristic of Alzheimer's disease (AD). The aim of the present study conducted on human neuronal SK-N-BE cells was to evaluate whether oligomerized A$\beta_{1\text{-}40}$-induced cell damages was associated with lipid modifications. Under treatment with A$\beta_{1\text{-}40}$ (10 - 100 µM; 24 - 48 h), cell viability was recorded with the MTT test and by measuring LDH activity. Mitochondrial transmembrane potential and ATP production were assessed using flow cytometry and a luciferase-based ATP bioluminescence assay, respectively. Annexin V-CF647 staining assay for cell apoptosis detection was performed using flow cytometry. Potentially intracellular cytotoxic lipids (oxysterols: 7α-hydroxycholesterol (7α-OHC), 7β-hydroxycholesterol (7β-OHC), and 7-ketocholesterol (7KC), 24(S)-hydroxycholesterol; arachidonic acid (C20:4 n-6); VLCFAs (C22:0, C24:0, C24:6 and C26:0)) were measured using gas chromatography coupled with mass spectrometry. The cellular level of docosahexaenoic acid (C22:6 n-3), often altered in AD, was also quantified. In the presence of A$\beta_{1\text{-}40}$, the percentage of MTT-positive cells decreased and was associated with an increase in LDH activity. In addition, treatment with oligomerized A$\beta_{1\text{-}40}$ induced a decrease of mitochondrial transmembrane potential as well as an apoptotic cell death. Sterol analysis revealed a higher cholesterol level and a significant increase of cytotoxic oxysterols per cell (7KC + 7β-OHC), and of the [(7β-OHC + 7KC)/cholesterol] ratio, considered as a lipid peroxidation index, in A$\beta_{1\text{-}40}$-treated cells. An enhancement of C20:4 n-6, C22:6 n-3 and saturated VLCFAs was also observed. Therefore, A$\beta_{1\text{-}40}$-induced side ef-

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fects are associated with intracellular accumulation of lipids, especially cholesterol, oxysterols (7β-OHC, 7KC), C20:4 n-6, and saturated VLCFAs, which could in turn contribute to neurotoxicity.

Keywords

SK-N-BE Cells, Oligomerized A$\beta_{1\text{-}40}$, Cholesterol, Oxysterols, Very Long Chain Fatty Acids

1. Introduction

Alzheimer's disease (AD) is the most predominant dementia in the elderly. Aggregated amyloid deposits are the main components of senile plaques, which are characteristics of the AD brain [1]. Amyloid beta peptide (Aβ), known to trigger numerous types of neuronal damages, is generated by sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretase [2].

At the moment, AD has been associated with several risk factors, and among them lipid alterations have been suspected [3] [4]. The γ-secretase cleavage site, which is directly centered within the transmembrane domain, suggests that membrane composition, especially the lipid environment, may influence Aβ generation [5]. Furthermore, numerous studies support the notion that an alteration of cholesterol metabolism and cholesterol oxide production, particularly 24(S)-hydroxycholesterol (24S-OHC), 27-hydroxycholesterol (27-OHC), 7KC, and 7β-OHC, can play critical roles in degenerative diseases such as AD [3] [4] [6]. Currently, the relationship between hypercholesterolemia and dementia is not clearly understood [7]. In humans, it is however well established that the APOE polymorphism and in particular the presence of the ε4 isoform is associated with a greater risk of developing AD [8]. In contrast to cholesterol, several arguments suggest that oxysterols probably play critical roles in AD. Increased levels of 7KC and 7β-OHC, resulting from autoxidation of cholesterol during oxidative stress [9], have been shown in brain lesions [10] as well as an increase in both 27-OHC and 24S-OHC in the frontal cortex of AD patients [11]. In addition, enhanced plasma levels of 24S-OHC, which could be a consequence of neuronal damages, have been reported during the first stages of dementia [12]. However, decreased plasma levels of 24S-OHC were reported in other stages and were associated with cerebral atrophy and severity of dementia [13]. It should be noted that 7KC, 7β-OHC, and 24S-OHC are potent inducers of cell death and also have pro-oxidant and pro-inflammatory activities on numerous cells, including those of the central nervous system [14]. Furthermore, it was reported that 24S-OHC downregulates APP trafficking resulting in suppression of Aβ production [15]. In contrast, 27-OHC enhances production of A$\beta_{1\text{-}42}$ by up-regulating APP and β-secretase [16].

Another finding relating lipid metabolism disorders to AD pathogenesis was the accumulation of saturated very long chain fatty acids (saturated VLCFAs: docosanoic acid (C22:0), tetracosanoic acid (C24:0) and hexacosanoic acid (C26:0)) in cortical regions of brains of AD patients with stages V-VI compared with those modestly affected (stages I-II) based on the neuropathological Braak classification [17]. In addition, in the plasma of demented patients, including AD patients, a marked accumulation of C26:0 was observed [18]. This fatty acid accumulation, in particular C24:0 and C26:0, was suspected of being the consequence of peroxisomal dysfunctions since these VLCFAs are metabolized in the peroxisome by β-oxidation [19]. In agreement with the possible alteration of peroxisomal metabolism suspected in AD, modifications of docosahexaenoic acid (DHA, C22:6 n-3) and plasmalogen levels were reported [19]. These various observations indicate substantial lipid alterations in AD, which may contribute to the initiation and/or progression of the disease.

With important roles attributed to Aβ in the development of AD due to its multiple neurotoxic activities [20], and since major lipid modifications involving increased levels of cholesterol and neurotoxic lipids (oxysterols: 7KC, 7β-OHC, 24S-OHC; saturated VLCFAs: C22:0, C24:0, C26:0) can be observed in the brain, the cerebrospinal fluid, and/or the plasma of AD patients [4] [17] [18], it was of interest to determine the ability of Aβ to induce lipid disorders on neuronal cells. So, the present study was realized on human neuroblastoma SK-N-BE cells to simultaneously evaluate the cytotoxic activity of the oligomerized A$\beta_{1\text{-}40}$ and its ability to trigger lipid alterations. Cell viability was recorded with the MTT test, quantification of ATP level and LDH activity, and measurement of mitochondrial transmembrane potential with $DiOC_6(3)$. The induction of apoptosis was evaluated with Annexin V. The impact on cholesterol, oxysterols, and fatty acid levels was determined using gas

chromatography coupled with mass spectrometry.

2. Material and Methods

2.1. Cells and Cell Treatments

As previously described, human neuronal cells (SK-N-BE) were seeded at 200,000 cells per well in 24-well microplates containing 1 mL of culture medium constituted by Dulbecco's Modified Eagle Medium with L-glutamine (DMEM) (Lonza) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Pan Biotech) and 1% antibiotics (100 U/mL penicillin, 100 mg/mL streptomycin) (Pan Biotech) [21]. A$\beta_{1\text{-}40}$ peptide (Sigma Aldrich) was solubilized in 1 mL of phosphate buffered saline exempt of calcium and incubated for 7 days at 37˚C.

2.2. Evaluation of Mitochondrial Activity with the Colorimetric MTT Assay

The MTT assay was carried as previously described [21] on SK-N-BE cells plated in 24-well flat-bottom culture plates with oligomeric A$\beta_{1\text{-}40}$ (10 and/or 100 µM, 48 h). The MTT assay was used to evaluate the effects of oligomeric A$\beta_{1\text{-}40}$ on mitochondrial activity and/or cell growth. Indeed, the tetrazolium salt (MTT) is reduced to formazan in the metabolic active cells by mitochondrial succinate dehydrogenase. A microplate reader was used to record mitochondrial activity and/or cell growth at a wavelength of 570 nm.

2.3. LDH Release Assay

Cytotoxicity induced by A$\beta_{1\text{-}40}$ was assessed by lactate dehydrogenase (LDH) leakage into the culture medium. The LDH activity was determined using a commercially available kit (Cayman Chemical Company). The assay is based on the conversion of lactate to pyruvate in the presence of LDH with parallel reduction of NAD. NADH formed from the above reaction is used by diaphorase to catalyze the reduction of tetrazolium salt to formazan which is proportional to the quantity of LDH released in the medium. A microplate reader was used at a wavelength of 490 nm and LDH activity was determined from the calibration curve. This LDH activity was adjusted to the number of cells per well, and was expressed as µU/mg of protein.

2.4. Intracellular ATP Measurement

Intracellular ATP levels were measured using a luciferase-based ATP Bioluminescence Assay Kit CLS II (Roche Molecular Biochemicals). For ATP measurement, 100 µL of cell lysate was mixed with 50 µL of luciferase. Emitted bioluminescence was measured using a microplate reader. The protein of each treatment group was determined by the BCA Protein Assay Kit.

2.5. Flow Cytometric Measurement of Transmembrane Mitochondrial Potential with $DiOC_6$ (3)

Variations of the transmembrane mitochondrial potential (ΔΨm) were measured with 3, 3'-dihexyloxacarbocyanine iodide ($DiOC_6$ (3)) (Invitrogen), which allows the percentage of cells with low ΔΨm to be determined. With $DiOC_6$ (3), mitochondrial depolarization is indicated by a decrease in green fluorescence collected through a 520/10-nm band pass filter. $DiOC_6$ (3) was used at a 40 nM. Flow cytometric analyses were performed on a Galaxy flow cytometer (Partec). Ten thousand cells were acquired for each sample. Data were analyzed with Flomax software (Partec) or FlowJo software (Tree Star Inc.).

2.6. Cell Apoptosis Analysis

Apoptotic cell death was measured via Annexin V-CF647 (Millipore) staining followed by flow cytometry. Annexin V is a calcium-dependent phospholipid binding protein with high affinity for phosphatidylserine (PS), a membrane component normally localized to the internal face of the cell membrane and which is exposed on the cell surface upon induction of apoptosis. Annexin V, which is conjugated to CF647 (Abs/Em maxima: 650/665 nm), was excited by a red laser on a FACSalibur 4C flow cytometer (BD Biosciences) and the emission of fluorescence was collected with a 670 nm long pass filter. Five µL Annexin V-CF647 were added to the cells in the dark at 37˚C, in a humidified atmosphere containing 5% CO_2. Around 15 min later, the stained cells were ana-

lyzed by flow cytometry. Ten thousand cells were acquired for each sample. Data were analyzed with Flomax software (Partec) or FlowJo software (Tree Star Inc.).

2.7. Quantification of Cholesterol, Cholesterol Oxide Derivatives and Fatty Acids by Gas Chromatography Coupled with Mass Spectrometry

Cholesterol oxide derivatives (also called oxysterols), including 7α-OHC (mainly formed via CYP7A1 [22] but which can also arise from the decomposition of 7α-hydroperoxycholesterol produced by free radical oxidation of cholesterol [23]), those oxidized at C7 resulting from cholesterol autoxidation (7KC and 7β-OHC) [9], as well as 24S-OHC, and cholesterol were quantified as follow. After trypsinization, cells were suspended in ethanol containing butylated hydroxytoluene (Sigma; 50 µg/mL) and EDTA (Sigma; 50 µg/mL). 7β-OHC (d7) (Avanti Polar lipids/Coger), 24S-OHC (d6) (Avanti Polar lipids/Coger), and Epicoprostanol (Sigma) were added as internal standards. Samples were then subjected to alkaline hydrolysis with 0.35 M KOH for 2 h at room temperature. The reaction mixture was adjusted to pH 7 with phosphoric acid, and lipids were extracted with hexane. After solvent evaporation, 100 µL of a mixture of N, O-bis (trimethylsilyl) trifluoroacetamide, and trimethylchlorosilane (4/1, v/v) (Acros Organics, Fisher Scientific) were added, and samples were incubated at 80˚C for 60 min to form trimethylsilyl ethers. After evaporation, the residue was dissolved in 100 µL hexane for gas chromatography coupled with mass spectrometry (GC-MS) analysis. GC-MS was performed using an Agilent Technology 6890 GC equipped with an HP7683 injector and a 5973 mass selective detector (Agilent Technologies). Chromatography was performed using a HP-5MS-fused silica capillary column (length: 25 m; inner diameter: 0.25 mm; film thickness: 0.25 µm; Agilent Technologies). GC-MS conditions were as follows: carrier gas, helium at a flow-rate of 1.1 mL/min; injector temperature, 250˚C; oven temperature, 180˚C increased at 10˚C/min to 260˚C, then at 1˚C/min to 280˚C and held for 5 min. The mass spectrometer was operated in the electron impact mode with an electron energy of 70 eV. The ion source temperature and the quadrupole temperature were 230˚C and 150˚C, respectively. The ions used for analysis were 24S-OHC 145 *m/z*, 24S-OHC (d6) 151 *m/z*, (25-OHC) 131 *m/z*, cholesterol 368 *m/z*, epicoprostanol 370 *m/z*, 7α-OHC 456 *m/z*, 7β-OHC 456 *m/z*, 7β-OHC (d7) 463 *m/z*, and 7KC 472 *m/z*. Calibration curves were obtained using authentic standards extracted with the method used for cell samples.

C22:0, C24:0, C26:0, C20:4, C22:6, and C24:6 were quantified using a HP7890A gas chromatograph equipped with an HP7683 injector and a HP5975C mass selective detector (Agilent Technologies). Chromatography was performed using an HP-5MS-fused silica capillary column (length: 30 m; inner diameter: 0.25 mm; film thickness: 0.25 mm; Agilent Technologies). The GC-MS conditions were as follows: carrier gas, helium at a flow rate of 1.1 mL/min; injector temperature, 250˚C, split mode; oven temperature, 140˚C increased at 5˚C/min to 300˚C and held for 10 min. The mass spectrometer was operated under negative chemical ionization mode with methane as the reactant gas. The ion source temperature and the quadrupole temperature were 150˚C and 106˚C, respectively. A SIM program was used for mass spectrometry with [M-181] (−) ions as quantification.

3. Results

3.1. Effect of A$\beta_{1\text{-}40}$ on Cell Viability

The ability of A$\beta_{1\text{-}40}$ to induce neurotoxicity was estimated using i) the MTT test, which reflects mitochondrial activity and/or cell growth, and ii) by LDH activity. SK-NB-E cells were cultured without or with A$\beta_{1\text{-}40}$ (10 - 100 µM, 24 - 48 h). A significant decrease in the percentage of MTT-positive cells was observed after 24 and 48 h of treatment with the two concentrations of A$\beta_{1\text{-}40}$ used (**Figure 1(a)**). With A$\beta_{1\text{-}40}$ (10-100 µM), as cytotoxic effects in the same range of order were observed with the MTT test, LDH activity was only measured on SK-N-BE cells treated with A$\beta_{1\text{-}40}$ (10 µM). An increase in LDH activity was observed after 24 - 48 h of treatment. However, significant differences between control (untreated cells) and A$\beta_{1\text{-}40}$-treated cells were only found at 48 h (**Figure 1(b)**).

3.2. Effect of A$\beta_{1\text{-}40}$ on ATP Production and Transmembrane Mitochondrial Potential

Data obtained with the MTT test support that mitochondrial activity and/or cell growth is affected under treatment

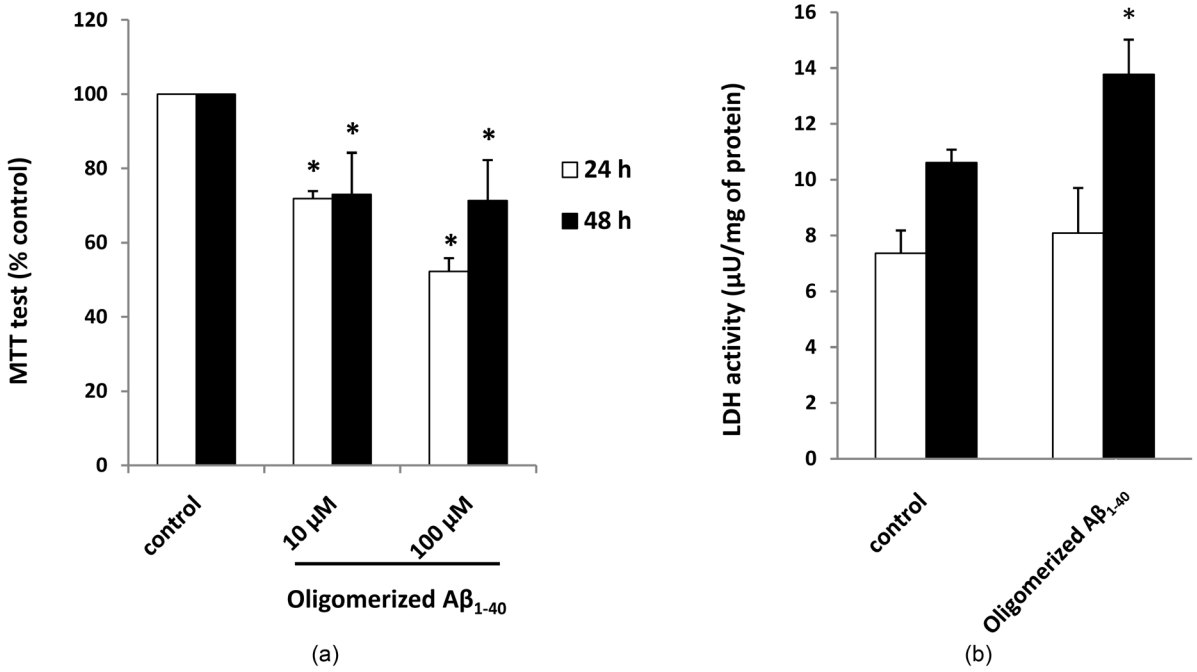

Figure 1. Effects of oligomerized A$\beta_{1\text{-}40}$ on cell viability. SK-N-BE cells were incubated with or without A$\beta_{1\text{-}40}$ (10 - 100 µM) for 24 and/or 48 h. Cell proliferation and/or mitochondrial metabolism was evaluated using the MTT test (a) and cell death by LDH activity (b). Data shown are mean ± SD from two to three separate experiments conducted in triplicate. Significance of the difference is indicated by * (Mann-Whitney test; $^{*}P < 0.05$).

with A$\beta_{1\text{-}40}$. To determine the impact of A$\beta_{1\text{-}40}$ at the mitochondrial level, ATP production and mitochondrial transmembrane potential ($\Delta\Psi$m) were measured. A significant increase of the percentage of $DiOC_6$ (3) negative cells (with low $\Delta\Psi$m) was observed with 100 µM at 24 h, and with 10 and 100 µM at 48 h of treatment with oligomerized A$\beta_{1\text{-}40}$ (**Figure 2(a)**).

The ATP level was measured on SK-N-BE cells treated with oligomerized A$\beta_{1\text{-}40}$ (10 µM, 48 h). A significant increase in intracellular ATP supporting mitochondrial dysfunctions was revealed in treated cells compared to the control (**Figure 2(b)**).

3.3. Effect of A$\beta_{1\text{-}40}$ on Apoptotic Cell Death Induction

Annexin V-CF647 staining assay for cell apoptosis detection was performed using flow cytometry. A significant increase of the percentage of Annexin V positive cells was observed in cells treated with oligomerized A$\beta_{1\text{-}40}$ (100 µM, 24 h) and with 10 and 100 µM at 48 h ($P < 0.05$) (**Figure 3**).

3.4. Effect of A$\beta_{1\text{-}40}$ on Lipid Profile

The effects of A$\beta_{1\text{-}40}$ (10 µM, 48 h) on the intracellular levels of cholesterol, oxysterols (7α-OHC, 7β-OHC, 7KC, 24S-OHC) (**Table 1**), and fatty acids (C20:4 n-6 (AA), C22:0, C22:6 n-3 (DHA), C24:0, C24:6) (**Table 2**) was investigated using GC-MS on SK-N-BE cells. Substantial modifications of the intracellular levels of cholesterol and oxysterols were observed (**Table 1**).

Cholesterol analysis revealed a significant increase (Mann-Whitney test; $P < 0.05$) in A$\beta_{1\text{-}40}$-treated cells. In addition, oxysterol analysis in SK-N-BE-treated cells revealed a significant increase (Mann-Whitney test; $P < 0.05$) in 7β-OHC and in the sum (7β-OHC + 7KC), reflecting cholesterol autoxidation. Furthermore, the [(7β-OHC + 7KC)/cholesterol] ratio, considered as a lipid peroxidation index, was significantly enhanced (Mann-Whitney test; $P < 0.05$) in A$\beta_{1\text{-}40}$-treated cells. However, no significant difference in the sum of cytotoxic oxysterols (7α-OHC + 7β-OHC + 7KC + 24S-OHC) was observed between untreated cells (control) and A$\beta_{1\text{-}40}$-treated cells, whereas it was highest under treatment with A$\beta_{1\text{-}40}$.

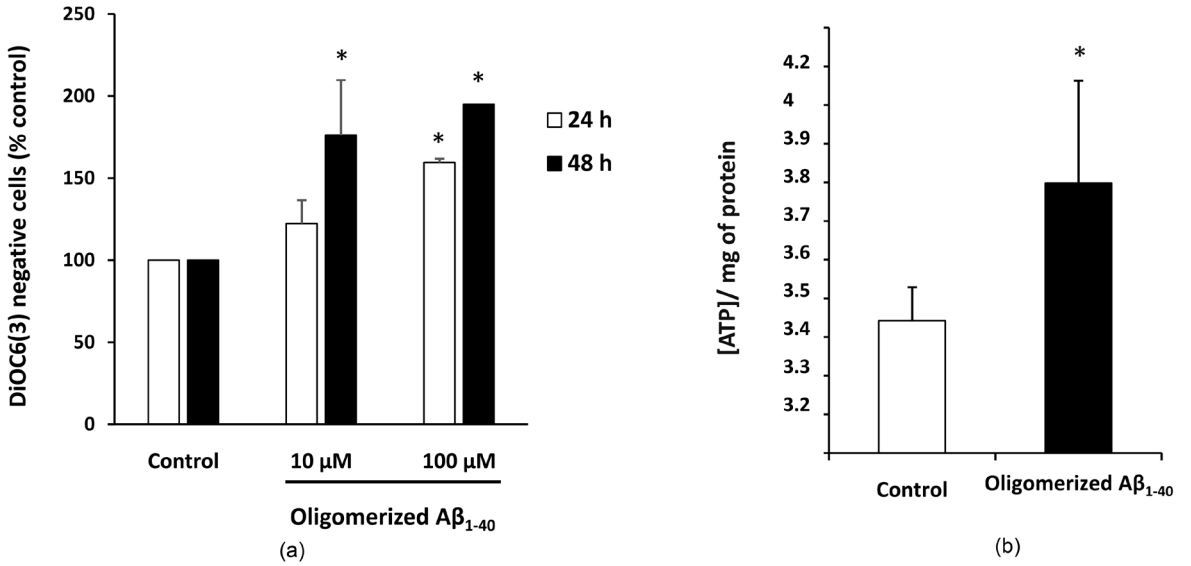

Figure 2. Effects of oligomerized A$\beta_{1\text{-}40}$ on transmembrane mitochondrial potential and ATP production. SK-N-BE cells were incubated without (control) or with A$\beta_{1\text{-}40}$ (10 µM, 48 h). (a): effect on transmembrane mitochondrial potential measured by flow cytometry with DiOC6 (3); (b): effect of oligomerized A$\beta_{1\text{-}40}$ (10 µM, 48 h) on ATP level measured using a luciferase-based ATP bioluminescence assay. Data shown are mean ± SD from two or three separate experiments conducted in triplicate. Significance of the difference is indicated by * (Mann-Whitney test; $^*P < 0.05$).

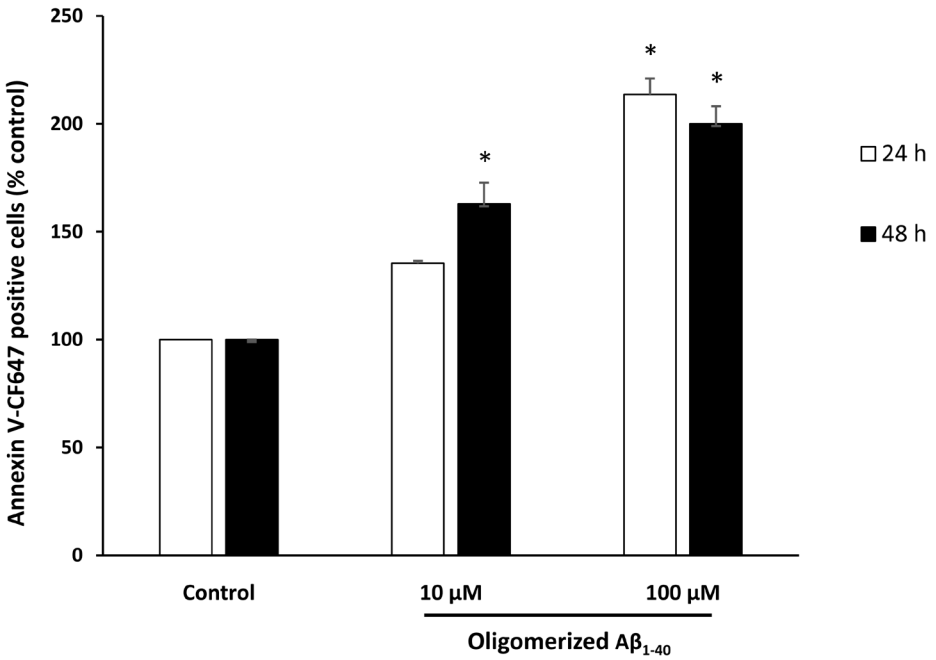

Figure 3. Effects of oligomerized A$\beta_{1\text{-}40}$ on apoptosis induction. SK-N-BE cells were incubated without (control) or with A$\beta_{1\text{-}40}$ (10 and 100 µM, 24 and 48 h). Cells were stained with Annexin V-CF647 and analyzed by flow cytometry. Data shown are mean ± SD from two or three separate experiments conducted in triplicate. Significance of the difference is indicated by * (Mann-Whitney test; $^*P < 0.05$).

Considerable modifications in the intracellular levels of fatty acids were also revealed (**Table 2**). Under treatment with A$\beta_{1\text{-}40}$ significant accumulations (Mann-Whitney test; $P < 0.05$) of AA (C20:4 n-6), C22:0, DHA (C22:6 n-3), and the sum of saturated VLCFAs (C22:0 + C24:0 + C26:0) was observed.

Table 1. Evaluation of the effects of oligomerized A$\beta_{1\text{-}40}$ on oxysterols profile.

	Cholesterol (pmoles/cell) $\times 10^{-6}$	**7α-OHC (pmoles/cell) $\times 10^{-6}$**	**7β-OHC (pmoles/cell) $\times 10^{-6}$**	**7KC (pmoles/cell) $\times 10^{-6}$**	**24S-OHC (pmoles/cell) $\times 10^{-6}$**	**7β-OHC+7KC (pmoles/cell) $\times 10^{-6}$**	**Σ oxysterols (pmoles/cell) $\times 10^{-6}$**	**(7β-OHC+7KC)/Cholesterol $\times 10^{-3}$**
Control (48 h)	7360.00 ± 385.00	1.97 ± 0.47	2.50 ± 0.34	7.89 ± 0.01	0.66 ± 0.14	10.40 ± 0.46	13.00 ± 0.98	1.41 ± 0.02
Oligomerized A$\beta_{1\text{-}40}$ (10 µM, 48 h)	8120.00* ± 133.00	2.08 ± 0.38	3.50* ± 0.28	8.93 ± 1.37	0.78 ± 0.12	12.40* ± 1.66	15.30 ± 1.93	1.53* ± 0.02

SK-NB-E cells were cultured for 48 h without (control) or with A$\beta_{1\text{-}40}$ (10 µM; 48 h). Cholesterol and oxysterols analysis was realized by GC-MS. Data (expressed in pmoles/cell) are mean ± SD. Significance of the difference is indicated by *(Mann-Whitney test; $^{*}P < 0.05$). Σ oxysterols: (7α-OHC + 7β-OHC + 7KC + 24S-OHC).

Table 2. Evaluation of the effects of oligomerized A$\beta_{1\text{-}40}$ on fatty acids profile.

	C20:4 n-6 (nmoles/cell) $\times 10^{-6}$	**C22:0 (nmoles/cell) $\times 10^{-6}$**	**C22:6 n-3 (nmoles/cell) $\times 10^{-6}$**	**C24:0 (nmoles/cell) $\times 10^{-6}$**	**C24:6 (nmoles/cell) $\times 10^{-6}$**	**C26:0 (nmoles/cell) $\times 10^{-6}$**	**(C22:0 + C24:0 + C26:0) (nmoles/cell) $\times 10^{-6}$**
Control (48 h)	1.530 ± 0.180	0.016 ± 0.002	0.250 ± 0.044	0.014 ± 0.002	0.027 ± 0.005	0.007 ± 0.001	0.037 ± 0.005
Oligomerized A$\beta_{1\text{-}40}$ (10 µM, 48 h)	8.110* ± 0.180	0.020* ± 0.001	0.370* ± 0.070	0.018 ± 0.003	0.032 ± 0.003	0.009 ± 0.002	0.047* ± 0.006

SK-NB-E cells were cultured for 48 h without (control) or with A$\beta_{1\text{-}40}$ (10 µM; 48 h). Fatty acids analysis was realized by GC-MS. Data (expressed in nmoles/cell) are mean ± SD. Significance of the difference is indicated by *(Mann-Whitney test; $^{*}P < 0.05$).

4. Discussion

Amyloid peptide (Aβ), the main component of senile plaques, was shown to be neurotoxic in several studies but no data are available to evaluate the relation between this molecule and lipid metabolism disorders associated with AD pathogenesis [7] [14] [18]. To attain a better understanding of the neurotoxicity of A$\beta_{1\text{-}40}$, its effects on the cellular lipid profile were considered. On human neuronal SK-N-BE cells, our data show that A$\beta_{1\text{-}40}$ favors the accumulation of cholesterol, oxysterols, and fatty acids, which are known to play critical roles in the development of AD [14].

On SK-N-BE cells, the neurotoxicity of oligomerized A$\beta_{1\text{-}40}$ evaluated with the MTT test and LDH activity showed both a significant increase in the percentage of MTT-positive cells and an increase in LDH activity, which supports the ability of A$\beta_{1\text{-}40}$ to induce cell death [24]. This decrease of mitochondrial succinate dehydrogenase activity associated to the loss of transmembrane mitochondrial potential observed after staining with $DiOC_6$ (3) suggests that A$\beta_{1\text{-}40}$ can induce mitochondrial alterations that are assumed to contribute to the pathogenesis of AD [25]. The oligomerized A$\beta_{1\text{-}40}$, as its isomer the A$\beta_{1\text{-}42}$, was able to induce apoptotic cell death in SK-N-BE cells evaluated by PS externalization revealed with Annexin V. A$\beta_{1\text{-}42}$ also induces apoptosis in cultured FVB mouse hippocampal neurons [26].

The significant increase in intracellular ATP observed in SK-N-BE cells treated with A$\beta_{1\text{-}40}$ supports the hypothesis that stressed cells may require more energy to counteract various side effects resulting from stress conditions and to preserve their vital functions [27]. This adaptive response of neural cells to an environmental stress could also explain (at least in part) the ability of A$\beta_{1\text{-}40}$ to disturb lipid homeostasia.

The increased intracellular level of cholesterol detected in A$\beta_{1\text{-}40}$-treated cells supports the notion that the cellular stress triggered by A$\beta_{1\text{-}40}$ can promote cholesterol synthesis and/or accumulation [28]. There is also a great deal of evidence on cultured neurons [29] and in transgenic mouse models [30] suggesting that cholesterol accumulation is linked to Aβ. Recent evidence using mouse models of cholesterol loading demonstrates that cholesterol sensitizes neurons to Aβ-induced oxidant cell death [31]. As it is known that A$\beta_{1\text{-}40}$ is a pro-oxidant molecule [32], we determined the impact of A$\beta_{1\text{-}40}$ on lipid peroxidation via the generation of cholesterol oxide derivatives resulting from cholesterol autoxidation (7β-OHC and 7KC, mainly). Interestingly, a significant accumulation of 7β-OHC and (7β-OHC + 7KC) was detected. Moreover, the [(7β-OHC + 7KC)/cholesterol] ratio (considered as a lipid peroxidation index) was also significantly enhanced. These oxysterols produced in the cells through the autoxidation of cholesterol not only argue in favor of the occurrence of an oxidative stress induced by oligomerized A$\beta_{1\text{-}40}$, but this also provides information on the potential cytotoxic pathways adopted by oligomerized A$\beta_{1\text{-}40}$. Indeed, some oxysterols, mainly those oxidized at C7 (7α-OHC, 7β-OHC, 7KC), are cytotoxic and able to induce cell death associated with oxidative processes [14] [33]. Therefore, the oxysterols could in turn contribute to the cytotoxic effects of A$\beta_{1\text{-}40}$.

Although not significant, the enhancement of the intracellular level of 24S-OHC (a potent liver X receptor (LXR) agonist produced by enzymatic oxidation of cholesterol via CY46A1) [4] [14] may have negative consequences. It could contribute to disturb cholesterol level via LXR, and/or participate in the cytotoxic effects of A$\beta_{1\text{-}40}$. Indeed, it is well established that 24S-OHC has a wide range of activities that depend on its concentration.

On the other hand, analysis of intracellular fatty acids conducted on SK-N-BE cells treated with A$\beta_{1\text{-}40}$ revealed a significant increase of AA (C20:4 n-3), C22:0 and DHA (C22:6 n-3) and the sum of VLCFAs ((C22:0 + C24:0 + C26:0). These results underline that A$\beta_{1\text{-}40}$ could disrupt the metabolism of fatty acids and especially affect the peroxisomal β-oxidation of VLCFAs given that the β-oxidation or the synthesis of some of these lipids (C22:6 n-3, C24:0, and C26:0) occurs, at least in part, in the peroxisome [19]. As a cortical accumulation of C22:0, C24:0, and C26:0 has been found in patients with stages V and VI pathology compared with those modestly affected (stages I and II) based on the neuropathological Braak staging for AD patients [17], our data obtained on SK-N-BE cells support the hypothesis that A$\beta_{1\text{-}40}$ could favor peroxisomal dysfunctions leading to reduced peroxisomal β-oxidation, which could thus contribute to the development of AD, as previously suggested [17]. However, as a simultaneous increase in DHA produced by β-oxidation was simultaneously observed, it cannot be excluded that abnormal elongase activities could be also activated, and contribute to the accumulation of C22:0, C24:0, and C26:0 [19]. Nevertheless, the ability of A$\beta_{1\text{-}40}$ to favor the accumulation of VLCFAs, which are strong inducers of mitochondrial dysfunctions and trigger oxidative stress on various neuronal cells [21], reinforces the hypothesis that these fatty acids could constitute potential risk factors contributing to the

development of brain lesions in AD [19].

The important accumulation of arachidonic acid (AA), the precursor of leukotrienes and prostaglandins, on SK-N-BE cells treated with A$\beta_{1\text{-}40}$ also contributes new insights into the biological activities of this molecule. This finding is in agreement with data reporting that eicosanoids might participate in A$\beta_{1\text{-}40}$ toxicity in neurons and that noncytokinic inflammation contributes to the development of AD [34]. It is also known that AA can participate to neurotoxicity via its ability to decrease neuroprotectins [35]. However, recent findings also suggest that prostaglandin derived from AA might also have neuroprotective effects [36]. Therefore, the increase in AA could be an adaptive response that could either contribute to A$\beta_{1\text{-}40}$ cytotoxic effects or to counteract its side effects.

In addition to its ability to trigger cell death, our data establish that A$\beta_{1\text{-}40}$ favors a substantial cellular accumulation of lipids: cholesterol, oxysterols (especially those resulting from cholesterol autoxidation), and fatty acids. Since a marked accumulation of VLCFAs and DHA was observed, modifications of lipid metabolism, including peroxisomal dysfunctions, are suspected. It is suggested that the accumulation of cholesterol, oxysterols, and fatty acids could in turn contribute to the cytotoxic effects of A$\beta_{1\text{-}40}$. Consequently, the identification of molecules capable of counteracting the different side effects of these lipids may be advantageous in preventing the neurotoxicity induced by A$\beta_{1\text{-}40}$.

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