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# Multiple inhibitory effects of Alzheimer's peptide Aβ1–40 on lipid biosynthesis in cultured human HepG2 cells

Natalia V. Koudinova[a,c,*], Temirbolat T. Berezov[a,b], Alexei R. Koudinov[b]

[a]*Department of Biochemistry, Russian Peoples' Friendship University School of Medicine, Moscow, Russian Federation*
[b]*Russian Academy of Medical Sciences, Miklucho-Maklay St., 8, Moscow 117198, Russian Federation*
[c]*Department of Medicine, New York University Medical Center, 560 First Avenue, New York, NY 10016, USA*

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**Abstract Herein we describe the inhibitory effect of the synthetic peptide Aβ1–40, homologous to the major high-density lipoprotein-associated species of Alzheimer's amyloid β protein (Aβ), on lipid biosynthesis in human hepatic HepG2 cells. This culture synthesizes various lipids from [$^{14}$C]acetate as a precursor. Treatment of cells with different concentrations of Aβ1–40 decreased the syntheses of various radiolabeled lipid species. The decrease reached saturation at peptide concentrations equal to 10–100 ng ml$^{-1}$. The lipids whose synthesis was decreased most were free and esterified cholesterol and phospholipids. This inhibitory effect suggests that Aβ protein may modulate physiological intracellular lipid syntheses. It may also be of special importance in the pathological condition, and contribute to the neurodegeneration, in Alzheimer's disease and related disorders.**

*Key words:* Amyloid beta protein; Apolipoprotein; Alzheimer's disease; Down's syndrome; Lipid biosynthesis; Lipoprotein

## 1. Introduction

Amyloid β (Aβ), a proteolytic product of the amyloid precursor protein (βPP), is a hydrophobic peptide of 39–44 amino acids, which was long considered the only pathologic protein, composing the amyloid plaques and cerebrovascular amyloid in Alzheimer's disease (AD), Downs' syndrome (DS) and related disorders. Nevertheless, in 1992, it was found as a soluble component (sAβ) in plasma and cerebrospinal fluid (CSF) of both normal and AD patients, as well as in many cell cultures' supernatants (for a review see [1]). Furthermore, the Aβ sequence appears to be highly conserved in eukaryotes [2–4], while βPP mRNA is expressed in many species [1]. These facts suggest that Aβ is involved in some basic metabolic pathways.

Our studies yielded experimental evidence of sAβ association with high density lipoproteins (HDL) in both normal human plasma [5] and CSF [6,7]. These are the most dense lipoprotein complexes, which carry out important functions in lipid metabolism [8]. One of these functions is the crucial step of reverse cholesterol transport, the reaction of cholesterol esterification, catalyzed by lecithin-cholesterol acyltransferase (LCAT, EC 2.3.1.4.3) [9]. Association of sAβ with HDL suggests that it may have some function(s) in lipid metabolism, as do many other apolipoproteins [10]. This possibility is strengthened by our recent observation of the inhibition of normal human plasma cholesterol esterification by Alzheimer's peptides Aβ1–40 and Aβ1–28 [6,11].

All this prompted us to elucidate whether Aβ protein has other functions in lipid metabolism, in particular, whether it affects cellular lipid synthesis. Hepatic cells in culture have provided a useful model to study the effects of different compounds on lipid metabolism [12,13] without interference from the other tissues (vascular, adipose, etc.). In this paper, we report on the effects of Alzheimer's peptide Aβ1–40 on hepatic lipid syntheses using a hepatocellular carcinoma derived HepG2 cell line [14] as model. The results show that Aβ1–40 treatment decreases the synthesis of lipids, in particular free and esterified cholesterol and phospholipids, in HepG2 cells. These data may help further understanding of the normal biology of sAβ and the mechanism of neurodegeneration in Alzheimer's brain.

## 2. Materials and methods

All reagents unless otherwise specified were purchased from Sigma. Human serum albumin (HSA) was essentially fatty acid free. Petroleum ether was from Fisher Scientific. Synthetic peptide Aβ1–40 was synthesized at the W.M. Keck Facility at Yale University and repurified as previously described [11].

HepG2 human hepatocellular carcinoma cell line was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown on 6-well cell culture plates (35 mm diameter well) in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS) and 6.99 mM L-glutamine. All cell cultivations and incubations were performed at 37°C in a humidified incubator with 5% $CO_2$-95% air. For passage, cells were harvested with 0.25% trypsin and 1 mM EDTA. Experiments were started when cell monolayers became 90% confluent. Cell morphology and ultrastructure were monitored by transmissional electron microscopy as previously described [15]. The absence of staining with fluorescein diacetate was used as the criterion for cell viability [16].

To assess lipid syntheses, cells in culture were labeled with 0.1 μCi [$^{14}$C]acetate (50 mCi/mmol, ICN, USA) per well in 0.5 ml of FBS-free medium [17]. Labeling was performed without Aβ peptide (control) and in the presence of the peptide at different concentrations (0.5, 1.0, 5.0, 10.0, 100.0 and 500.0 ng ml$^{-1}$). Labeling was also performed with HSA at the concentrations specified. After 3 h of incubation, the cultures were rinsed three times with 2 ml of FBS-free medium and re-fed with 0.75 ml of medium without radioactive precursor. After 24 h incubation, the monolayers were washed three times with cold phosphate-buffered saline, pH 7.3 and the intracellular lipids were extracted and analyzed as described below.

Extraction of intracellular lipids was performed directly from cell monolayers on the 6-well plates. Intracellular lipids were extracted three times for 30 min at 25°C with 300 μl of hexane/isopropanol

*Corresponding author. Department of Biochemistry, Russian Peoples' Friendship University School of Medicine, Miklucho-Maklay St. 8, Moscow 117198, Russia. Fax: (7) (095) 246-2690/246-7446, c/o Dmitri B. Kalugin. E-mail: koudin@imb.imb.ac.ru

*Abbreviations:* Aβ, amyloid beta; AD, Alzheimer's disease; CSF, cerebrospinal fluid; DS, Down's syndrome; FBS, fetal bovine serum; HDL, high density lipoprotein; HSA, human serum albumin; sAβ, soluble form of Aβ; TLC, thin-layer chromatography


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(3:2, v/v) [18]. The solvent phases were combined and then dried under a stream of nitrogen and resuspended in 50 μl of hexane.

Extracted intracellular lipids were separated by thin-layer chromatography (TLC) [19] on silica gel G TLC plates (Whatmann, USA) in a solvent system of petroleum ether-diethyl ether-acetic acid (70:30:1, v/v) and located by exposure to iodine vapor. The areas corresponding to authentic lipid standards were placed in scintillation vials with 2 ml of BetaMax scintillation fluid (ICN, USA) and counted.

For protein content determination, cell monolayers after lipid extractions were solubilized in 0.5 ml/well of 0.2 N NaOH for 16 h at room temperature [20]. Protein content in aliquots of solubilized cells was quantitated using a commercially available kit from Bio-Rad. Bovine serum albumin, 2 mg/ml water solution (Pierce, USA), was used as a standard for the calibration curve.

The intracellular synthesis of each lipid class (phospholipids, unesterified and esterified cholesterol, and triacylglycerols) was expressed as lipid-incorporated $^{14}C$ radioactivity (cpm) per μg of cell protein and represents mean ± SEM, $n$=9. Each experimental point was a mean of four repeats. For the tested concentrations of Aβ1–40 and HSA the data were expressed as a percentage of control (no peptide/protein) change in $^{14}C$ radioactivity (cpm) incorporation into specific lipid classes per μg of cell protein.

Non-parametric Wilcoxon signed rank test was used for determining significant differences between lipid synthesis in the presence of Aβ1–40 and without it [21]. A probability of 0.05 or less was accepted as statistically significant.

## 3. Results and discussion

To assess whether Aβ protein affects cellular lipid synthesis we used hepatic HepG2 cells. These are cells commonly used for lipid and lipoprotein metabolism and lipid synthesis studies [14,22]. Intracellular lipid synthesis was traced by the incorporation of [$^{14}C$]acetate radioactivity into the newly synthesized phospholipids, free and esterified cholesterol and triacylglycerol in the presence of different amounts of Aβ1–40, shown to be the major form of HDL-associated sAβ protein [6,7]. The control values (no peptide added) for [$^{14}C$]acetate radioactivity incorporation (cpm/μg of cell protein) into different lipid classes under our experimental conditions were: 18.54 ± 0.45 for phospholipids, 0.954 ± 0.059 and 0.534 ± 0.025 for free and esterified cholesterol, respectively, and 7.63 ± 0.42 for triacylglycerol.

Aβ1–40 inhibited multiple intracellular lipid syntheses, in particular the syntheses of phospholipids (Fig. 1A), free and esterified cholesterol (Fig. 1B), and triacylglycerol (Fig. 1C), while HSA used as a control did not affect the syntheses within the tested concentration range. The inhibitory effect was concentration dependent and reached saturation at 10–100 ng of the peptide per ml of culture media, although cytotoxic effects were not found for any tested Aβ1–40 concentration within the incubation period of 3 h. The inhibitory effects of Aβ1–40 were also statistically significant at concentrations lower than 10 ng $ml^{-1}$. This may well reflect physiological modulation of lipid metabolism by Aβ protein and represent one of the lipid related functions of sAβ as an apolipoprotein constituent of HDL [5–7]. On the other hand, the high concentrations of Aβ peptide (100 and 500 ng $ml^{-1}$) also studied herein are not likely to be reached under physiological conditions, but may well represent the local tissue environment in AD brain. If the inhibitory effects of Aβ protein, reported here in the model of HepG2 cells of hepatic origin, similarly take place in the brain tissue, it would suggest an explanation of the reported changes in lipid composition in specific brain regions in AD [23–25]. In addition, the differences in the effects of Aβ on the metabolism of unesterified cholesterol (40% maximum inhibition, Fig. 1B) and phospholipid (25% maximum inhibition, Fig. 1A) would clarify the cholesterol to phospholipid mole ratio decrease in the AD temporal gyrus versus age-matched controls, observed previously [26]. Our data thus extend current knowledge [23–26], providing a possible biochemical explanation for membrane destabilization secondary to a lipid compositional aberration as a mechanism for Aβ-dependent neurodegeneration in AD.

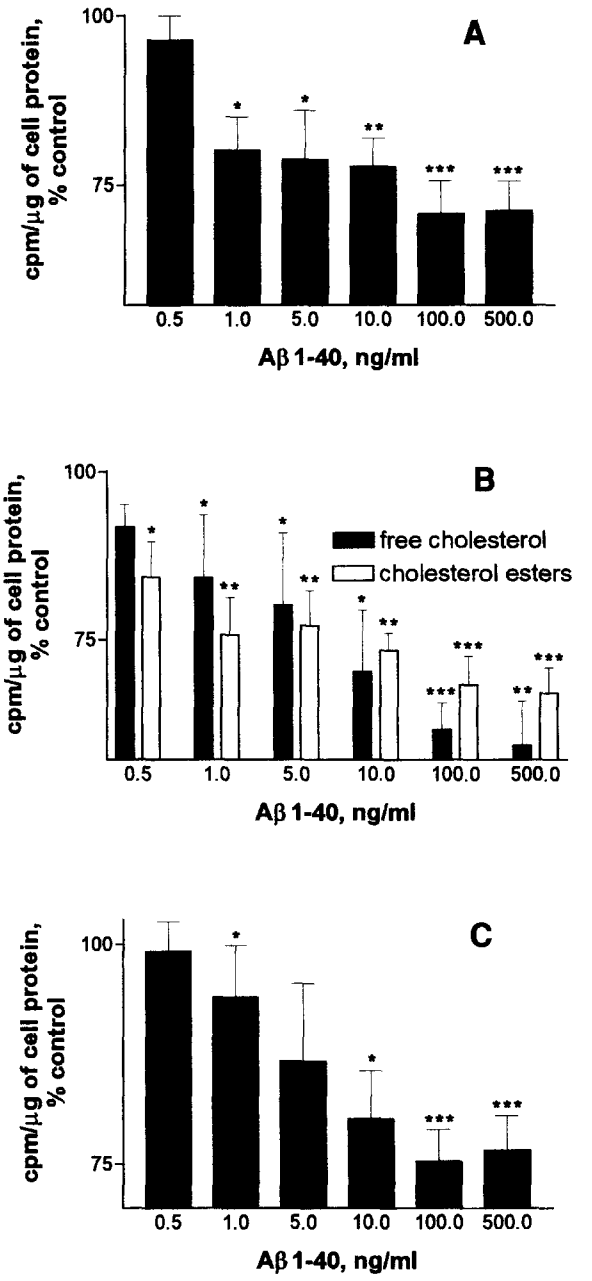


Fig. 1. Effect of Aβ1–40 on the incorporation of [$^{14}C$]acetate into phospholipids (A), cholesterol (B), and triacylglycerols (C) in HepG2 cells. The incorporation of [$^{14}C$]acetate was determined after extraction of lipids and their separation by TLC. Values are mean ± SEM, $n$ = 9. Each experimental point is the mean of four repeats. Asterisks indicate significant differences with respect to control (no peptide added) values (100%): *$P$ < 0.02; **$P$ < 0.008; ***$P$ < 0.001, one-tailed. HSA as a control did not affect the incorporation of [$^{14}C$]acetate into HepG2 lipids.

Nevertheless, the question of what is the primary event for

this purported cascade remains obscure. Induction of Aβ immunoreactivity in the brains of rabbits with enriched dietary cholesterol [27] suggests, assuming the concentration-dependent inhibitory effect of Aβ protein on lipid biosynthesis reported here, that an increase in Aβ concentration within the affected brain tissue might be secondary, and due to prior changes in lipid metabolism, particularly of cholesterol. Such induction of Aβ deposition [27] may also be the case in subjects, carrying the E4/E4 allele of apoE, shown to have elevated plasma cholesterol levels [28,29].

Our results show that Aβ1–40 reduced the incorporation of [$^{14}$C]acetate into newly synthesized intracellular lipids of cultured HepG2 cells. Since this is a steady-state measurement, the observed effect may also be due to Aβ1–40 mediated increase in lipid catabolism. Further studies are needed to clarify the actual mechanism of such an Aβ effect. This is especially important in terms of reported increase of phospholipid catabolism in AD brain tissue [30]. Further discussion of Aβ involvement in the lipid turnover and the other consequences of sAβ association with HDL will be forthcoming.

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