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Methylglyoxal reduces mitochondrial potential and activates Bax and caspase-3 in neurons: Implications for Alzheimer's disease

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HIGHLIGHTS

- Amyloid induces methylglyoxal production.
- Tyr modification of TPI is the cause of methylglyoxal production.
- Methylglyoxal triggers neuronal apoptosis.

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ABSTRACT

Alzheimer's disease (AD) is characterized by the oxidative stress generated from amyloid β-peptide (Aβ) aggregates. It produces protein nitrotyrosination, after the reaction with nitric oxide to form peroxynitrite, being triosephosphate isomerase (TPI) one of the most affected proteins. TPI is a glycolytic enzyme that catalyzes the interconversion between glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP). Methylglyoxal (MG) is a by-product of TPI activity whose production is triggered when TPI is nitrotyrosinated. MG is harmful to cells because it glycates proteins. Here we found protein glycation when human neuroblastoma cells were treated with Aβ. Moreover glycation was also observed when neuroblastoma cells overexpressed mutated TPI where Tyr165 or Tyr209, the two tyrosines close to the catalytic center, were changed by Phe in order to mimic the effect of nitrotyrosination. The pathological relevance of these findings was studied by challenging cells with Aβ oligomers and MG. A significant decrease in mitochondrial transmembrane potential, one of the first apoptotic events, was obtained. Therefore, increasing concentrations of MG were assayed searching for MG effect in neuronal apoptosis. We found a decrease of the protective Bcl2 and an increase of the proapoptotic caspase-3 and Bax levels. Our results suggest that MG is triggering apoptosis in neurons and it would play a key role in AD neurodegeneration.

1. Introduction

Alzheimer's disease is characterized by the aggregation into beta-sheets of amyloid β-peptide (Aβ); which produces neuronal death. Aβ reduces Cu^{2+} and Fe^{2+} and produces H_2O_2 [1,2] which will generate a downstream intracellular cascade of reactive oxygen species (ROS). Therefore superoxide anion ($O_2^{\bullet -}$) reacts with nitric oxide (NO) to form the highly reactive peroxynitrite anion (ONOO—); which will nitrate the tyrosine residues of the protein in a process termed nitrotyrosination. This modification is an irreversible reaction that consists of the addition of a nitro group (NO_2) to a tyrosine residue; generating 3-nitrotyrosine [3,4]. Nitrotyrosination is a pathological event associated with several neurodegenerative diseases [5] and in particular with AD [6–11].

Abbreviations: Ab, antibody; Aβ, amyloid β-peptide; AD, Alzheimer disease; AGEs, advanced glycation end-products; DHAP, dihydroxyacetone phosphate; FBS, fetal bovine serum; GAP, D-glyceraldehyde-3-phosphate; H_2O_2, hydrogen peroxide; MG, methylglyoxal; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NO, nitric oxide; nNOS, neuronal NO synthase; o.e., overexpressing; TPI, triosephosphate isomerase; WT, Wild type.

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One of the proteins more nitrotyrosinated in AD is the glycolytic enzyme triosephosphate isomerase (TPI) [6–8,11].

TPI is a key enzyme in cell metabolism that controls the glycolytic flow and energy production catalyzing the interconversion of D-glyceraldehyde-3-phosphate (GAP) to dihydroxyacetone phosphate (DHAP) [12]. Any interruption of glycolysis causes brain dysfunction and memory loss, favoring neurodegeneration [13]. TPI is the only glycolytic enzyme whose functional deficiency is associated with neurodegeneration [14] and has been related to reduced longevity [15]. A by-product of the isomerase activity is methylglyoxal (MG), a harmful compound that induces protein glycation [16].

The present work showed the relevance of glycation in AD, proposing MG production as an effector of deleterious consequences of Aβ toxicity, demonstrating that MG can upregulate the proapoptotic signaling.

2. Material and methods

2.1. Cell lines

The human neuroblastoma cell line (SH-SY5Y) was grown in Ham's F12 medium (Invitrogen, Carlsbad, USA) supplemented with 15% FBS and 1% streptomycin/penicillin at 37 °C in a humidified atmosphere containing 5% CO_2.

2.2. $A\beta_{1-42}$ oligomer preparation

Synthetic $A\beta_{1-42}$ (EZBiolab, Carmel, USA) oligomers were obtained by dissolving 300 μg freeze-dried aliquots in 20 μL DMSO. Peptide stock aliquots were diluted in 0.1 M Tris–HCl at pH 7.4 to a final concentration of 88.6 μM Aβ. Solutions were stirred continuously at 37 °C and 300 rpm for 3 h and kept at −80 °C before being used.

2.3. Immunofluorescence study of Aβ treatment

Cells were seeded on poly-L-lysine coated coverslips in 24-well plates at a density of 50,000 cells/500 μL/well. After 12 h the medium was removed and Ham's F12 without FBS was added to the wells. Then cells were treated with 1 μM Aβ oligomers. After 24 h, cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. SH-SY5Y cells were immunostained with 1:100 mouse monoclonal anti-argypirimidine Ab (Cosmo Bio Co., Ltd., Carslbad, USA) and 1:2000 Alexa 555-bound as secondary Ab (Sigma, St. Louis, USA) at room temperature. Coverslips were mounted and analyzed using a Leica TCS SP confocal microscope and analyzed with Leica confocal software.

2.4. Study of the overexpression of TPI

TPI was amplified by PCR from purified human chromosomal DNA and cloned into a construct containing a 5′ upstream flag sequence. Wild-type (WT) TPI and TPI carrying a tyrosine mutation (Y165F or Y209F) were produced and subcloned into a pcDNA3 plasmid. SH-SY5Y cells were seeded in 6-well plates at a density of 5×10^5 cells per well and grown for 24 h with Ham's F12 medium supplemented with 15% FBS. Afterwards, 2 μg per well of each construct was transfected using Lipofectamine 2000 Reagent (Invitrogen, Carlsbad, USA), following manufacturer instructions. After 3 h, the medium was replaced by DMEM plus 10% FBS and 1% penicillin/streptomycin. Cells were incubated for 24 h before their lysis to proceed with western blot assays.

2.5. Measurement of $\Delta\Psi_m$

SH-SY5Y cells were seeded onto 60 mm-diameter-dishes at a density of 700,000 cells/3 mL/dish. After 12 h the medium was removed and Ham's F12 without FBS was added to the wells. Then cells were treated with 1 μM Aβ oligomers or 500 μM MG. After 24 h cells were re-suspended and mixed to reach a concentration of 10^6 cells/mL in PBS with the fluorophore MitoTracker Red CMXRos (Molecular Probes, Carlsbad, USA). Cells were incubated for 10 min at 37 °C and analyzed in a FACScan (Becton Dickinson, CA, USA).

2.6. Cell viability assays

SH-SY5Y cells were seeded in 96-well plates at a density of 25,000 cells/200 μL. Cells were treated win increasing concentrations of MG and incubated 22 h. Then 11 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) stock solution (5 mg/mL) were added per well; after 2 h the reaction was stopped with 120 μL of DMSO. MTT reduction was determined in a plate reader spectrophotometer at 540 and 650 nm. Control cells were taken as 100%.

2.7. Regulation of Bcl-2, Bax and caspase-3 study

SH-SY5Y cells were seeded onto 60 mm-diameter-dishes at a density of 700,000 cells/4 mL per dish. After 24 h in culture, cells were treated with increasing concentrations of MG (100, 250 and 500 μM) (Sigma). Cells were incubated with MG for 24 h before their lysis to proceed with western blot assays.

2.8. Western blot procedure

Cells were lysed on ice with a solution containing 1 M Tris–HCl, 1% Nonidet P-40, 150 mM NaCl, 5 mM EDTA, 1 mM sodium orthovanadate, 1 mM dithiotreitol, pH 7.4, and a protease inhibitor cocktail. Protein concentration was determined by Bradford assay. Aliquots were run in electrophoresis gels and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were incubated over night at 4 °C with mouse anti-argpyrimidine monoclonal Ab (1:1000; Cosmo Bio Co., Ltd.), rabbit anti-cleaved caspase-3 (Asp175, Cell Signaling, Beverly, USA) Ab, rabbit anti-Bax Ab (1:1000; Cell Signaling, Beverly, USA), rabbit anti-Bcl-2 Ab (1:1000; Cell Signaling, Beverly, USA), or mouse anti-β-actin monoclonal Ab (1:5000, Sigma, St. Louis, USA). Membranes were incubated with peroxidase-conjugated secondary Ab (1:3000; GE-Healthcare, UK) for 1 h at RT. Bands were visualized using the enhanced chemiluminescence substrate (Super Signal; Pierce).

2.9. Statistical analysis

Data were expressed as the mean ± SEM of the values from the number of experiments as indicated in the corresponding figures. Data were evaluated by Student *t*-test.

3. Results

3.1. Aβ induces protein glycation

We have previously demonstrated that brain samples from AD patients have increased protein glycation as well as double transgenic mice o.e. human amyloid precursor protein bearing the mutation Swedish and presenilin 1 [11]. Here we challenged human neuroblastoma cells with Aβ oligomers to demonstrate an increase of protein glycation due to Aβ action by immunofluorescence techniques (Fig. 1A).