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Vitamin D_3 protects against Aβ peptide cytotoxicity in differentiated human neuroblastoma SH- SY5Y cells: A role for S1P1/p38MAPK/ATF4 axis

Federica Pierucci [a, b], Mercedes Garcia-Gil [c, d], Alessia Frati [a], Francesca Bini [a], Maria Martinesi [a], Eleonora Vannini [e], Marco Mainardi [e], Federico Luzzati [f], Paolo Peretto [f], Matteo Caleo [e], Elisabetta Meacci [a, b, *]

[a] Department of Experimental and Clinical Biomedical Sciences "Mario Serio", Molecular and Applied Biology Research Unit, University of Florence, Viale GB Morgagni 50, 5134 Firenze, Italy
[b] Interuniversitary Miology Institutes, Italy
[c] Department of Biology, University of Pisa, Via San Zen, 31, 56127 Pisa, Italy
[d] Interdepartmental Research Center Nutrafood "Nutraceuticals and Food for Health", University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy
[e] Neuroscience Institute, CNR, 56124 Pisa, Italy
[f] Department of Life Sciences and Systems Biology, University of Turin, Neuroscience Institute Cavalier Ottolenghi, Via Verdi 8, 10124 Turin, Italy

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ABSTRACT

Besides its classical function of bone metabolism regulation, 1alpha, 25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$), acts on a variety of tissues including the nervous system, where the hormone plays an important role as neuroprotective, antiproliferating and differentiating agent. Sphingolipids are bioactive lipids that play critical and complex roles in regulating cell fate. In the present paper we have investigated whether sphingolipids are involved in the protective action of $1,25(OH)_2D_3$. We have found that $1,25(OH)_2D_3$ prevents amyloid-β peptide (Aβ(1–42)) cytotoxicity both in differentiated SH-SY5Y human neuroblastoma cells and *in vivo*. In differentiated SH-SY5Y cells, Aβ(1–42) strongly reduces the sphingosine-1-phosphate (S1P)/ceramide (Cer) ratio while $1,25(OH)_2D_3$ partially reverts this effect. $1,25(OH)_2D_3$ reverts also the Aβ(1–42)-induced reduction of sphingosine kinase activity. We have also studied the crosstalk between $1,25(OH)_2D_3$ and S1P signaling pathways downstream to the activation of S1P receptor subtype S1P1. Notably, we found that $1,25(OH)_2D_3$ prevents the reduction of S1P1 expression promoted by Aβ(1–42) and thereby it modulates the downstream signaling leading to ER stress damage (p38MAPK/ATF4). Similar effects were observed by using ZK191784. In addition, chronic treatment with $1,25(OH)_2D_3$ protects from aggregated Aβ(1–42)-induced damage in the CA1 region of the rat hippocampus and promotes cell proliferation in the hippocampal dentate gyrus of adult mice.

In conclusion, these results represent the first evidence of the role of $1,25(OH)_2D_3$ and its structural analogue ZK191784 in counteracting the Aβ(1–42) peptide-induced toxicity through the modulation of S1P/S1P1/p38MAPK/ATF4 pathway in differentiated SH-SY5Y cells.

1. Introduction

The amyloid-β peptide (Aβ) is the main constituent of amyloid plaques, and it is believed to play a causative role in the neurodegenerative process occurring in Alzheimer's disease (AD). The molecular mechanism underlying Aβ toxicity remains largely undefined although accumulating evidence indicates that the binding of hydrophobic Aβ assemblies to cellular membranes triggers multiple effects affecting diverse pathways (Selkoe and Hardy, 2016). Aβ toxicity has been shown to be linked with sphingolipid (SL) metabolism in both cell culture models and animal models (Haughey, 2010; Grimm et al., 2013). SLs constitute a biologically active lipid class that is significantly important from

* Corresponding author. University of Florence, Department of Experimental and Clinical Biomedical Sciences "Mario Serio", Research unit of Molecular and Applied Biology, Viale GB Morgagni 50, 50134 Firenze, Italy.
E-mail address: elisabetta.meacci@unifi.it (E. Meacci).

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both structural and regulatory aspects (Ghasemi et al., 2016; Pyne et al., 2016; Gomez-Muñoz et al., 2016). Indeed, they regulate fundamental cellular processes that are important in determining cellular fate, such as proliferation, apoptosis, cell cycle arrest, senescence, and inflammation (Airola and Hannun, 2013; Maceyka and Spiegel, 2014). Cells maintain a dynamic balance of distinct SL metabolites, with ceramide (Cer) and sphingoid bases serving as activators of cell death pathways, whereas sphingosine-1-phosphate (S1P) primarily exerts mitogenic effects acting as intracellular mediator or as ligand of specific S1P receptors (S1PR) (Strub et al., 2010). Of note, it has been proposed that cell fate is regulated by the ratio between S1P and Cer/sphingosine (Sph), with a high ratio promoting cell survival or proliferation, and a low ratio inducing growth arrest or cell death (Cuvillier et al., 1996). Both S1P synthesis and S1PR expression are required for embryonic neurogenesis (Mizugishi et al., 2005), whereas in the adult nervous system, S1P/S1PR axis regulates neurotransmission, promotes survival and affects differentiation (Milstien et al., 2007).

Sphingomyelins (SM) decrease and Cers increase in AD (reviewed in Haughey, 2010; Walter and van Echten-Deckert, 2013) with prominent changes in the very long-chain C24:0 and C24:1 species. He et al. (2010) have also demonstrated an increase in Sph level and a decrease of S1P in AD brains compared with age-matched neurologically normal control subjects. More recently, it has been demonstrated that early in AD pathogenesis and prior to AD diagnosis, the activity of sphingosine kinase (SphK) activity, the enzyme responsible of S1P synthesis is reduced, while the activity of the enzyme responsible for S1P degradation, S1P lyase, is increased (Ceccom et al., 2014; Couttas et al., 2014). Moreover, silencing SphK1 in an animal model of AD accelerates Aβ deposition and aggravates the memory deficit (Zhang et al., 2013). Changes in SL metabolites have been reported either in experiments in which Aβ has been applied exogenously or when Aβ was produced endogenously. In particular, SH-SY5Y neuroblastoma cells incubated with Aβ display a marked down-regulation of SphK1 activity coupled with an increase in the ceramide/S1P ratio followed by cell death (Gomez-Brouchet et al., 2007).

Besides its classical function of bone metabolism regulation, $1,25(OH)_2D_3$ acts on a variety of tissues including nervous system, where it has neuroprotective, antiproliferating and differentiating effects (Berridge, 2015; DeLuca et al., 2013). Since these processes are also regulated by SLs, these bioactive lipids could be mediators of $1,25(OH)_2D_3$ action in neural cells. On this line, we have recently demonstrated that ceramide kinase (CerK), the enzyme involved in Cer phosphorylation, plays a crucial role in the antiproliferative effects of $1,25(OH)_2D_3$ in human neuroblastoma cells (Bini et al., 2012). Deficit of $1,25(OH)_2D_3$ and several polymorphisms of $1,25(OH)_2D_3$ receptor (VDR) have been associated with AD risk (Łaczmański et al., 2015; Annweiler et al., 2015). Moreover, basic research and epidemiological studies have reported that $1,25(OH)_2D_3$ plays a protective role against impaired biological processes associated with AD and reduced cognition, such as those promoted by the toxic peptide.

In this paper, we aimed to investigate the potential involvement of SLs in the neuroprotective action of $1,25(OH)_2D_3$ against Aβ(1–42) toxicity in differentiated SH-SY5Y cells as well as in animal models. SH-SY5Y cells, induced to differentiate by incubation with retinoic acid and BDNF, were used to dissect the potential crosstalk between SLs and $1,25(OH)_2D_3$ in preventing the neurotoxicity induced by Aβ(1–42). To evaluate the *in vivo* neuroprotective effects, we used intracerebral administration of Aβ(1–42). Specifically, we examined the impact of systemic administration of $1,25(OH)_2D_3$ on hippocampal subgranular zone of the dentate gyrus (SGZ) and subventricular zone (SVZ) neurogenesis. Since previous studies have clearly shown that Aβ delivery to the rat hippocampus induces neuronal loss (Stepanichev et al., 2003; Xuan et al., 2012), in the CA1 pyramidal cell layer the $1,25(OH)_2D_3$-mediated neuroprotection against Aβ(1–42) was also evaluated. Interestingly, we found that $1,25(OH)_2D_3$ counteracts Aβ(1–42) toxicity through the axis S1P/S1P1 receptor/p38MAPK/ATF4 in SH-SY5Y cells; on the other hand, Aβ(1–42) injection prevented neurogenesis induced by the hormone in SGZ area.

2. Materials and methods

2.1. Materials

Biochemicals, cell culture reagents, Dulbecco's Modified Eagle's Medium (DMEM), fetal calf serum (FCS), penicillin/streptomycin, protease inhibitor cocktail, and bovine serum albumin, were purchased from Sigma (St. Louis, MO, USA) except phenol red-free Ham's F-12 medium that was from BioSource (Camarillo, CA, USA); SH-SY5Y cells were obtained from American Type Culture Collection (ATCC, Manassa, VA, USA); Lyophilized $A\beta_{1-42}$ was purchased from Sigma (Milan, Italy); D-erythro- Sphingosine 1-phosphate was from Calbiochem (San Diego, CA,USA); $1,25(OH)_2D_3$ was from Sigma, Syber Green reagent was from Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA); Chemioluminiscence kit was from GE Healthcare (Chalfont St. Giles, UK); CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit was from Promega (Madison, WI, USA); anti-phospho-p38 and total p38 antibodies from Cell Signaling (Danvers, MA, USA); anti-BAX, anti-ATF4, anti-β-actin antibodies and secondary antibodies conjugated to horse radish peroxidase were from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-S1P1 was from Sigma (Milan, Italy); anti guinea-pig IgG antibody conjugated with Alexa488 (Jackson ImmunoResearch Laboratories, Suffolk, UK). Guinea pig polyclonal antibody anti-NeuN were from (Millipore, Vimodrone, Italy). BrdU (rat IgG monoclonal, Bio-Rad Laboratories, Biotinylated antibodies were from Vector Labs, Burlingame, CA, USA); O.C.T. was from Tissue-Tek (Sakura Finetek USA, Inc. Torrance, CA USA); avidin-biotin-peroxidase kit was from Vector Labs (Vector Laboratories LTD, Peterborough, UK); [^{3}H]serine, [^{3}H]Sphingosine and [^{32}P]ATP (6000 Ci/mmol) were from Perkin Elmer (Monza, Italy). W146 was from Tocris Bioscience (Bristol, UK). ZK191784, and ZK159222 were kindly provided by Bayer Shering Pharma AG (Berlin, Germany); Yoyo-1, was from Life Technologies; ketamine was from Ketavet (Gellini, Aprilia, Italy) and xylazine (Rompun; Bayer AG, Leverkusen, Germany) Sintex (Nuova Chimica, Cinisello Balsamo, Italy).

2.1.1. Animals

Fully adult Long–Evans hooded rats (350 g) and adult (3 months-old) CD1 male mice bred in animal facilities at CNR (Pisa, Italy) and Neuroscience Institute Cavalieri Ottolenghi (Turin, Italy) were used in this study. Animals were housed in a 12 h light/dark cycle with food and water available *ad libitum*. All experimental procedures were in conformity to the European Communities Council Directive 86/609/EEC and were approved by the Italian Ministry of Health.

2.1.2. SH-SY5Y cell culture and treatments

SH-SY5Y cells were grown in DMEM/HAM's F12 supplemented with 10% heat-inactivated fetal bovine serum and 100 U/mL penicillin/streptomycin (Bini et al., 2012). The cells were maintained at 37 °C in a humidified incubator with 5% CO_2 and 95% air. Medium was changed every other day and the cells were split (0.25% trypsin, 0.53 mM EDTA solution) when they reached approximately 80% confluence. All treatments were performed for 24 h using cells at approximately 60–70% confluence. Differentiation was induced