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Coconut oil protects cortical neurons from amyloid beta toxicity by enhancing signaling of cell survival pathways

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ABSTRACT

Alzheimer's disease is a progressive neurodegenerative disease that has links with other conditions that can often be modified by dietary and life-style interventions. In particular, coconut oil has received attention as having potentially having benefits in lessening the cognitive deficits associated with Alzheimer's disease. In a recent report, we showed that neuron survival in cultures co-treated with coconut oil and Aβ was rescued compared to cultures exposed only to Aβ. Here we investigated treatment with Aβ for 1, 6 or 24 h followed by addition of coconut oil for a further 24 h, or treatment with coconut oil for 24 h followed by Aβ exposure for various periods. Neuronal survival and several cellular parameters (cleaved caspase 3, synaptophysin labeling and ROS) were assessed. In addition, the influence of these treatments on relevant signaling pathways was investigated with Western blotting. In terms of the treatment timing, our data indicated that coconut oil rescues cells pre-exposed to Aβ for 1 or 6 h, but is less effective when the pre-exposure has been 24 h. However, pretreatment with coconut oil prior to Aβ exposure showed the best outcomes. Treatment with octanoic or lauric acid also provided protection against Aβ, but was not as effective as the complete oil. The coconut oil treatment reduced the number of cells with cleaved caspase and ROS labeling, as well as rescuing the loss of synaptophysin labeling observed with Aβ treatment. Treatment with coconut oil, as well as octanoic, decanoic and lauric acids, resulted in a modest increase in ketone bodies compared to controls. The biochemical data suggest that Akt and ERK activation may contribute to the survival promoting influence of coconut oil. This was supported by observations that a PI3-Kinase inhibitor blocked the rescue effect of CoOil on Aβ amyloid toxicity. Further studies into the mechanisms of action of coconut oil and its constituent medium chain fatty acids are warranted.

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1. Introduction

Alzheimer's Disease (AD) is characterized by neurofibrillary tangles (NFTs) and deposition of beta amyloid (Aβ) in plaques in the brain, which can directly or indirectly influence synapses and neuronal survival with resulting alterations in behaviour and cognition (Mucke and Selkoe, 2012; Selkoe and Hardy, 2016; Singh et al., 2016). Although the pathophysiology of Aβ toxicity is not completely understood, a number of potential mechanism including oxidative stress, mitochondrial dysfunction, changes in membrane permeability along with alterations in various signaling pathways have all been suggested to play roles (eg., (Carrillo-Mora et al., 2014; Crews and Masliah, 2010; Majd et al., 2015)). There is currently no cure for Alzheimer's despite the intense research into possible therapeutic interventions.

An emerging area of research deals with the role of dietary supplementation as an approach to ameliorating deficits associated with aging and neurodegeneration (Williams et al., 2015). Alzheimer's disease has links with other conditions that can often be modified by dietary and life-style interventions (Erbguth and Himmerich, 2014; Fernando et al., 2015; Lei et al., 2016). In particular, coconut oil has received attention as having potentially having benefits in lessening the cognitive deficits associated with Alzheimer's disease (Cunningham, 2011; DeDea, 2012; Fernando et al., 2015; Nafar and Mearow, 2014). Coconut oil has a high percentage of medium chain triglycerides (MCTs), which can be rapidly metabolized to induce metabolic ketosis, wherein circulating ketone bodies could provide an alternative energy source in situations

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Abbreviations

Aβ	amyloid-beta peptide, 1-42
ACC	acetyl-coA-carboxylase
AICAR	5-Aminoimidazole-4-carboxyamide ribonucleoside, AMPK activator
AMPK	AMP-activated protein kinase
Akt	RAC serine/threonine kinase
CoOil	virgin coconut oil, cold pressed
CpC	compound C, AMPK inhibitor
DMSO	dimethyl sulfoxide
ERK	extracellular signal-regulated kinase; mitogen activated protein kinase p42/44
GSK3	glycogen synthase kinase 3
MAPLC3B	Microtubule associated protein Light Chain 3B
ROS	reactive oxygen species

where glucose metabolism or glucose uptake might be compromised (Costantini et al., 2008; Cunnane et al., 2016; Henderson, 2008). While the focus on MCTs and MCFA has been part of the rationale for the use of coconut oil, other components such as polyphenols may play a role in promoting neuronal health via antioxidant effects (Arunima and Rajamohan, 2014; Marina et al., 2009).

Our recent publication was the first report to show that treatment of cultured cortical neurons with coconut oil (CoOil) could influence survival (Nafar and Mearow, 2014). In that pilot study, we showed that neuron survival in cultures co-treated with CoOil and Aβ was rescued compared to cultures exposed only to Aβ. Aβ treatment of neurons resulted in decreased mitochondrial size and increased circularity and CoOil co-treatment attenuated these changes as well. Our objective in the current study was to further investigate the effects of CoOil on cortical neuronal responses to exposure to Aβ *in vitro*. In the earlier experiments, cultures were concomitantly treated with Aβ and CoOil. Here we investigated treatment with Aβ for 1, 6 or 24 h followed by addition of CoOil for a further 24 h, or treatment with CoOil for 24 h followed by Aβ exposure for various periods. Neuronal survival and several cellular parameters (cleaved caspase 3, synaptophysin labeling and ROS) were assessed. In addition, the influence of these treatments on relevant signaling pathways was investigated with Western blotting. In terms of the treatment timing, our data indicated that CoOil can rescue cells pre-exposed to Aβ for 1 or 6 h, but is less effective when the pre-exposure has been 24 h. However, pretreatment with CoOil prior to Aβ exposure showed the best outcomes. The CoOil treatment reduced the number of cells with cleaved caspase and ROS labeling, as well as rescuing the loss of synaptophysin labeling observed with Aβ treatment. The biochemical data suggest that AMPK, Akt and ERK activation may contribute to the survival promoting influence of CoOil. Further studies into the mechanisms of action of CoOil and its constituent medium chain fatty acids are warranted.

2. Methods

2.1. Cultures

Cortical neuronal cultures were prepared from postnatal day 1 (P1) rats. Animal procedures were approved by the Animal Care Committee at Memorial University of Newfoundland in accordance with The Canadian Council on Animal Care (CCAC). Cortices were quickly dissected from isolated brains and placed in cold Hanks Balanced Salt Solution (HBSS + 10 mM HEPES, Gibco, Invitrogen, Burlington, Ont) (King et al., 2009; Nafar and Mearow, 2014). Meninges were removed and the tissue chopped into small pieces and dissociated enzymatically (0.125% Trypsin with 0.02% EDTA, Gibco, Invitrogen) for 15 min at 37 °C. Following enzyme inhibition, cells were then rinsed and triturated in Dulbeccos minimum essential medium (DMEM, Invitrogen Corp.), supplemented with 10% fetal calf serum (FCS), 20 mM KCl (Medium 1). Dissociated cells were collected by centrifugation, suspended in Medium 1 and counted using a haemocytometer. Cells were subsequently plated on poly-D-lysine (5 μg/ml) coated 12-well plates for western blot analysis or 16-well glass slides for neuronal survival and growth analysis. 2–4 h after plating Medium 1 was replaced with serum-free Neurobasal medium (NB, Gibco, Invitrogen) supplemented with 100 U/ml penicillin/streptomycin, 2% B-27 supplement and 1 mM Hepes. Typically, 1.5×10^6 cells/ml were plated for protein collection, whereas 50–100 μl of 2.0 10^5 cells/ml were plated for immunocytochemistry or survival assay experiments. At 2 div, cell culture media was supplemented to 10 mM cytosine arabinoside; all experimental treatments, unless otherwise specified were carried out at 5 div. With these culture conditions, cultures contain typically greater than 95% neurons.

Primary astrocyte cultures were prepared from neonatal rat cortices according to established methods with minor modifications (Mearow et al., 1990; Nafar et al., 2016); cultures were maintained in DMEM with 10% FCS and were used for experiments after the first passage. In our experience, these cultures are greater than 95% GFAP-positive astrocytes (Nafar et al., 2016). For the short term ketone assays, the medium was changed to either Neurobasal with no supplements or Hanks balanced salt solution for the duration of the assays (1–4 h).

2.2. Peptide preparation

Ultra pure amyloid peptide (1 mg, 1–42, rPeptide, Bogart, GA) was prepared according a modification of the method of Stine (Stine et al., 2003). Briefly lyophilized peptide was resuspended in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and incubated on a rotator until dissolved. The HFIP was evaporated under vacuum and the resulting film suspended in DMSO to make a 220 μM solution. The resulting peptide preparation consisted primarily of monomers with some dimers and trimers (4–12 kd) (Nafar et al., 2016; Stine et al., 2003). Amyloid peptide solution was then aliquoted, stored at −80 °C and thawed on ice before use.

2.3. Oil and fatty acid preparations

Approximately 2 g of virgin coconut oil (CoOil, Nutiva™ Organic Extra Virgin Coconut oil; cold-pressed and non-hydrogenated) was melted at 37 °C and a 1:1 emulsion in DMSO prepared. This emulsion was diluted to 0.1%, 0.01% and 0.001% in complete medium; all solutions/suspensions were subjected to further sonication and kept at 37 °C prior to addition to the cells. Octanoic (C:8, caprylic), Decanoic (C:10, capric) and Dodecanoic (C:12, lauric) acids were purchased from Sigma and suspended in either aqueous solution or in DMSO. The concentration of fatty acids used in our experiments was calculated based on an estimated concentration of 0.01% CoOil as 0.143 mM with approx 70% of this (0.1 mM) being MCFAs. Coconut oil contains approx 70% medium chain fatty acids (MCFAs), and of this, lauric acid comprises about 50%, caprylic acid (C:8) and capric (C:10) acid about 20%. We tested concentration of fatty acids ranging from 10 to 500 μM.

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