Coconut Oil Attenuates the Effects of Amyloid-β on Cortical Neurons in vitro

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Abstract. Dietary supplementation has been studied as an approach to ameliorating deficits associated with aging and neurodegeneration. We undertook this pilot study to investigate the effects of coconut oil supplementation directly on cortical neurons treated with amyloid-β (Aβ) peptide in vitro. Our results indicate that neuron survival in cultures co-treated with coconut oil and Aβ is rescued compared to cultures exposed only to Aβ. Coconut oil co-treatment also attenuates Aβ-induced mitochondrial alterations. The results of this pilot study provide a basis for further investigation of the effects of coconut oil, or its constituents, on neuronal survival focusing on mechanisms that may be involved.

Keywords: Coconut oil, Amyloid, cortical neurons

Dietary supplementation has been studied as an approach to ameliorating deficits associated with aging and neurodegeneration. In particular, recent anecdotal evidence has touted the use of coconut oil as having major benefits in lessening the cognitive deficits associated with Alzheimer’s disease (AD). Coconut oil has a high percentage of medium chain triglycerides (MCTs, ~70% of C:6-12), with caprylic and capric acid comprising ~21% of these MCTs in virgin coconut oil [1]. A clinical trial using a formulation of MCTs known as caprylidene (Ketasyn, AC-1202 caprylic acid) reported significant improvement in AD patients after 45 and 90 days of treatment [2]. This study led to the marketing of caprylidene as a ‘medical food’. However there has been much public interest in the use of over-the-counter coconut oil as a potential therapy, as it is less expensive and more widely available [3].

While there is rather limited scientific evidence that such treatment would have a significant effect on disease, there is scientific basis behind the use of MCTs, such as those found in coconut oils. MCTs can be rapidly metabolized to induce metabolic ketosis and ketogenic diets have been employed as therapy for a variety of brain disorders, including epilepsy and neurodegeneration [4–6]. Such diets lead to the formation of ketone bodies which can then be used as an alternative to glucose for energy requirements [4–7].

Experimental animal studies using dietary supplementation with MCT formulations or coconut oil have provided somewhat conflicting results. Those employing MCTs have reported positive effects on neuronal mitochondria function, energy metabolism, and decreases in amyloid-β protein precursor [8, 9]. In contrast, studies in which the diet has been supplemented with saturated fatty acids, particularly hydrogenated coconut oil, have reported deleterious effects on hippocampal morphology and behavior [10, 11]. These effects may have been related to the hydrogenated nature of the coconut oil.
In view of the interest in the potential of coconut oil as a dietary supplement that could ameliorate the symptoms of neurodegeneration, we undertook a pilot study to examine the influence of virgin coconut oil on rodent cortical neurons exposed to Aβ peptide.

Cortical neuronal cultures were prepared from postnatal day 1 (P1) rats (Sprague Dawley, Memorial University Animal Care Services) as previously described [12]. Animal procedures were approved by the Animal Care Committee at Memorial University of Newfoundland in accordance with The Canadian Council on Animal Care (CCAC). Ultra-pure amyloid peptide (1–42 or the reverse peptide control, rPeptide, Bogart, GA) was prepared according to the manufacturer’s instructions. 5 or 7.5 μM Aβ (or 1–40 scrambled peptide) was added to cultures at 5 DIV and incubated for varying periods of time (24–48 h) [12].

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. The CoOil solutions and the vehicle control (0.1% DMSO in complete medium) were added to the cells at the time of Aβ treatment and incubated for a further 24–48 h. Cells were then assessed for survival using MTT assays.

Neurons were incubated in MitoTracker Red (Molecular Probes, 200 nM in basal medium) for 30 min at 37°C, and subsequently processed for immunocytochemistry for MAP2 and neuron-specific β-tubulin as previously described [12]. Images were acquired by laser scanning confocal microscopy with sequential Z-stage scanning (Olympus FluoView 1000 confocal laser scanning microscope). Scanned stacks were compiled as individual images, and composite digital images were prepared in Adobe Photoshop CS. Mitochondrial parameters (area, sphericity, and volume) were analyzed using Imaris software (Bitplane Inc., South Windsor, CT). Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA) with significance determined by t-test or one way ANOVA and post-hoc testing using Tukey’s test.

Fig. 1A. There was a significant (p < 0.001) decrease in survival with 5 μM Aβ (82.6 ± 1.9%), and a further decrease with 7.5 μM (38.50 ± 3.9%). Treatment with CoOil suspensions (0.1%, 0.01%, or 0.001%) alone had no apparent deleterious effect on survival and, although not significant, there was a trend toward increased survival as shown in Fig. 1B.

When cultures were treated with 5 μM Aβ and the CoOil, there were no significant differences between the conditions (Fig. 1C). However, treatment of cells exposed to 7.5 μM Aβ with the CoOil resulted in significant increases in survival (Fig. 1D).

Cultures were treated for mitochondrial assessment and immunostaining as outlined above. Representative images of cultures showing labeling of mitochondria and immunostaining with beta III tubulin and MAP2 are shown in Fig. 2. Cultures treated with the CoOil and Aβ appeared to be healthier than those treated with Aβ alone (Fig. 2C, D, G, and H compared to Fig. 2B, F).

Data were acquired for mitochondrial volume (Fig. 2I and sphericity (Fig. 2J). Treatment of cultures with Aβ resulted in smaller mitochondria (Fig. 2I, p < 0.001), with an increased sphericity (Fig. 2J, p < 0.001). The presence of CoOil attenuated the changes that result from treatment with Aβ. Compared to Aβ alone, the area (not shown) and volume were significantly higher in the Aβ + CoOil (Fig. 2I). With 0.1% CoOil, there were no differences in any of the parameters compared to the vehicle control. At the lower concentrations of CoOil, the volume was decreased compared to control, but still higher than with the Aβ treatment.

Our results indicate that the presence of CoOil can ameliorate the toxic effects of Aβ on the neurons. Neuron survival in cultures co-treated with CoOil and Aβ is rescued compared to cultures exposed only to Aβ. Aβ treatment of neurons results in decreased mitochondrial size and increased circularity and CoOil co-treatment attenuates these changes as well. The rationale for using coconut oil as a potential AD therapy is related to the possibility that it could be metabolized to ketone bodies that would provide an alternative energy source for neurons, and thus compensate for mitochondrial dysfunction. However, there is little scientific evidence that coconut oil ingestion can improve cognition or other behavioral aspects in AD or other neurodegenerative diseases [3, 13].

While we did not directly investigate the contribution of specific CoOil fatty acids or polyphenols nor address any specific mechanisms in this pilot study, we found that in addition to ameliorating cell survival after Aβ treatment, there was also a positive effect of CoOil on mitochondrial size. Aβ exposure resulted in
Fig. 1. Effects of coconut oil treatment on the survival of cultured cortical neurons exposed to Aβ. Neuronal cultures were treated as outlined in the methods. Cellular survival was assessed using MTT assays (n = 6–8), and survival is presented as the percentage of the control vehicle-treated samples. A) Cultures exposed to vehicle control, 5 μM or 7.5 μM Aβ. **p < 0.001. B) Cultures exposed to different concentrations of oil (0.1, 0.01, 0.001%) show no difference from vehicle control. C) Cultures exposed to 5 μM Aβ plus coconut oil. D) Cultures exposed to 7.5 μM Aβ plus coconut oil. ** p < 0.001.

smaller and more spherical mitochondria, which could be a reflection of fragmentation or an alteration in mitochondrial fusion-fission dynamics [12, 14]. Mitochondrial dynamics and transport [15–17] are altered in AD. Aβ exposure has been shown to result in increased mitochondrial fission and decreased fusion [14, 18], and we have previously reported that Aβ resulted in decreased mitochondrial size and increased circularity in cortical neurons [12]. There have been no studies of the effects of coconut oil on these parameters, although there are several reports of the effect of D-β-hydroxybutyrate (the reduced form of ketones) on protection of neurons from Aβ. It was suggested that the beneficial effect of ketones may be to offset an Aβ-induced impairment of mitochondrial function and thus energy metabolism [19]. Other studies using MCT formulations derived from coconut oil (NeoBee™) have reported improvements in mitochondrial function and protection from age-related decreases in mitochondrial density [8, 9].

While the effect of dietary MCT supplementation seems to be linked primarily to the production of ketones and their use as an alternative energy source for the brain, the possibility of potential direct effects of MCTs on brain metabolism has been raised recently [20]. Caprylic acid (also known as octanoic acid and a major component of coconut oil) does cross the blood-brain barrier [20, 21]. A recent study examining the
Fig. 2. Effects of coconut oil treatment on cultures exposed to Aβ. Cultures were labeled with MitoTracker and then fixed for immunostaining with neuronal specific beta-III tubulin or MAP2. Mitochondria labeled with MitoTracker are shown in panels A–D; immunostaining of the same fields with neuronal specific tubulin and MAP2 are presented in panels E–H. A, E) vehicle control treated cultures; B, F) cultures treated with 7.5 μM Aβ for 24h; C, G) cultures treated with 0.1% coconut oil for 24h; D, H) cultures treated with both Aβ and 0.1% coconut oil for 24h. Quantification of mitochondrial volume and sphericity as outlined in the methods. ***p<0.001; **p<0.01; + significantly different from vehicle control, p<0.05. Scale bar: 20 μm.

The effect of MCTs on seizure prevention reported that caprylic acid exerted anti-convulsant effects independent of, and in addition to, the ketogenic effects [20]. On examination of our cultures, we noticed that there appeared to be more elongated mitochondria in astrocytes although we did not quantify these differences due to the difficulty in visually isolating glial cells from the surrounding neuronal networks. It is possible that the beneficial effect of the oil may be via the glial cells. MCTs are converted to ketones in the liver, but glial cells, in particular astrocytes, have also been reported to be able to metabolize MCTs to ketones that could be then shuttled to neurons for alternative energy [22, 23].

Another point to consider is the timing of the treatment. In the current study, neurons were treated concomitantly with the Aβ and the CoOil. It will be
important to assess whether pre-treatment with AβJ can be subsequently rescued by CoOil exposure, and whether there might be a particular time frame that would be optimal.

In summary, the results of this study provide a basis for further investigation of the effects of coconut oil or its constituents on neuronal survival, focusing on mechanisms that may be involved.

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