Crude caffeine reduces memory impairment and amyloid $\beta_{1-42}$ levels in an Alzheimer’s mouse model

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**A B S T R A C T**

Alzheimer’s disease (AD), a chronic neurodegenerative disorder associated with the abnormal accumulations of amyloid $\beta$ (A$\beta$) peptide and oxidative stress in the brain, is the most common form of dementia among the elderly. Crude caffeine (CC), a major by-product of the decaffeination of coffee, has potent hydrophilic antioxidant activity and may reduce inflammatory processes. Here, we showed that CC and pure caffeine intake had beneficial effects in a mouse model of AD. Administration of CC or pure caffeine for 2 months partially prevented memory impairment in AD mice, with CC having greater effects than pure caffeine. Furthermore, consumption of CC, but not pure caffeine, reduced the A$\beta_{1-42}$ levels and the number of amyloid plaques in the hippocampus. Moreover, CC and caffeine protected primary neurons from A$\beta$-induced cell death and suppressed A$\beta$-induced caspase-3 activity. Our data indicate that CC may contain propylactic agents against the cell death and the memory impairment in AD.

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1. Introduction

Alzheimer’s disease (AD) is a neurodegenerative disorder characterised by elevated levels of amyloid-$\beta$ (A$\beta$) in the brain and progressive cognitive impairment. Cleavage of amyloid precursor protein (APP) by the combination of $\beta$- and $\gamma$-secretases produces A$\beta$. A$\beta$ can aggregate into dimers, oligomers, and fibrils, and these aggregates can be toxic. A$\beta_{1-42}$ is one of the most toxic variants of A$\beta$, accumulates early in amyloid plaques, and aggregates more rapidly in vitro than A$\beta_{1-40}$ (Tanzi & Bertram, 2005). The accumulation of A$\beta$ in the brain induces neurodegenerative processes that have several mechanisms, including the initiation of free radical chain reactions (Butterfield & Boyd-Kimball, 2005). Oxidative macromolecules can be detected as early as the mild cognitive impairment (MCI) stage of the disease, suggesting a deficit in antioxidant capacity occurs early in the progression of AD (Guidi et al., 2006). Furthermore, A$\beta$ can cause inflammation and induce neuroinflammatory cytokine in brains of those with AD (Fiala & Veerhuis, 2010), suggesting that A$\beta$-mediated oxidative stress and inflammatory processes may be involved in the earliest events in the pathogenesis of AD.

Intake of caffeine, the most consumed natural psychoactive stimulant, is associated with a lower incidence of cognitive impairment in different neurological conditions, including sleep deprivation, alcohol consumption, diabetes, Parkinson’s disease, and AD (Alhaidar, Aleisa, Tran, Alzoubi, & Alkadihi, 2010; Duarte, Carvalho, Cunha, & Gruetter, 2009; Gevaerd, Takahashi, Silveira, & Da Cunha, 2001; Spinetta et al., 2008). In humans, studies have shown that caffeine intake is inversely correlated with cognitive decline and reduces depression (Ritchie et al., 2007; Smith, 2009). The combination of glucose and caffeine intake showed synergistic effects in improving learning and the consolidation of verbal memory (Adan & Serra-Grabulosa, 2010). In vitro and in vivo studies demonstrated that caffeine treatment protects cortical neurons against...
Aβ toxicity and prevents Aβ-induced memory deficits in mice (Arendash et al., 2006). Moreover, caffeine treatment reversed pre-existing memory impairment and reduced Aβ deposition in aged AD transgenic mice (Arendash et al., 2009). Caffeine prevents Aβ-induced synaptotoxicity and neuronal damage by acting as an antagonist of the adenosine A2A receptor (Canas et al., 2009). A long-term study in mice showed the protective effect of caffeine intake in the prevention of age-related memory decline, this effect was associated with changes in a neurotrophic factor (BDNF) (Costa et al., 2008). Moreover, caffeine and other components in coffee may synergize to protect against memory decline and AD (Cao et al., 2011). However, the identity of the coffee component that synergizes with caffeine is unknown.

Crude caffeine is the major by-product of the coffee decaffeination process, with thousands of tons of crude caffeine produced annually. In addition to caffeine, crude caffeine contains other bioactive components including caffeic acid, coumaric acid, vanillic acid, quercetin, and catechin. These coffee components have chemical and biological activities that can alleviate the physiological inflammatory states that lead to type 2 diabetes and neurodegenerative disorders (Chu et al., 2009, 2011). We have previously shown that crude caffeine contains higher levels of both hydrophilic and lipophilic antioxidant activity than pure caffeine (Chu et al., 2012). We also found that crude caffeine inhibited an indicator of inflammation, cyclooxygenase-2 (COX-2) (Chu et al., 2012). These activities of crude caffeine may be beneficial in the protection and/or intervention against AD. Thus, in this study, we investigated whether crude caffeine intake can protect against memory impairment and suppress brain Aβ1-42 levels in an AD mouse model.

2. Materials and methods

2.1. Animal model of Alzheimer's disease

The J20 mouse line, an AD mouse model that expresses the human APP gene with the Swedish (K670N/M671L) and Indiana (V717F) familial mutations, was obtained from The Jackson Laboratory (stock number 006293) and maintained on the C57BL/6j background. The experimental mice were housed in the Yang–Ming Laboratory Animal Center (National Yang–Ming University, Taiwan) in a specific pathogen free facility that had a light–dark cycle of 12 h light, 12 h dark. Male mice were used for this study and they had access to food and water ad libitum. All the animal studies were approved by the Institutional Animal Care and Use Committee of National Yang–Ming University. All animal diets were special or-dered from Harlan Laboratories (USA), including control diet (Tek-lad lab animal diets, global 16% protein diet, 2016, Harlan), CC diet (2016 + 0.0395% CC), or caffeine diet (2016 + 0.0375% caffeine). At the age of 3 months old, mice started to feed on these special diets until the time the mice were sacrificed (ages of sacrifice for all groups were listed in Table 1). Behavioural tests were performed 2 months after the experimental special diet was begun. Two days after all the behavioural tests, all mice were anaesthetised by the intraperitoneal injection of 2 mg/g body weight urethane (U2500, SIGMA, USA), and perfused by saline (0.9% NaCl) for 5 min after anaesthesia. Their brains were cut in half sagittally. One half was fixed for 48 h in 4% paraformaldehyde for plaque staining, and the other was snap-frozen for Aβ measurement.

2.2. Crude caffeine

Crude caffeine, a byproduct of the scCO2 decaffeination process detailed below, was obtained from Maximus Coffee Group (Houston, TX). Briefly, green coffee beans were soaked in water until the moisture content was 50%. Caffeine was removed in an extractor by liquid carbon dioxide at high temperature (90–100 °C) and high pressure (300 atm). The liquid carbon dioxide was recirculated between the extractor and a scrubber, where caffeine was removed from the liquid carbon dioxide with water. The resulting caffeine-rich aqueous solution was then concentrated by reverse osmosis and vacuum-dried. The proximate composition of the crude caffeine was analysed by Siliker, Inc. (South Holland, IL). The composition of crude caffeine and a biochemical comparison of pure and crude caffeine were reported in our previous study. Briefly, the crude caffeine contains 95.95% caffeine, 1.10% moisture, 1.04% fat, and 0.10% ash, manifesting a hydrophilic oxygen radical absorbance capacity (ORAC) of 145 ± 10 μmol Trolox equivalent (TE)/g and a lipophilic ORAC of 66 ± 7 μmol TE/g (Chu et al., 2012).

2.3. Water maze

The water maze paradigm was performed in a circular pool (diameter 1.2 m) filled with water. For the hidden platform test, a circular platform (diameter 12 cm) was submerged 1 cm below the water’s surface in the centre of the target quadrant. During the 5-day hidden platform test (6 trials per day), mice were randomly placed into one of the five starting point of the pool in each daily session. Mice were allowed to search for the platform for 60 s. If the mice did not find the platform within 60 s, they were gently guided to it and left on the platform for 5–10 s. The probe trial was performed 1 day after the last day of hidden platform test. The circular platform used in the hidden platform test was removed from the pool for the probe trial. The starting point was in the quadrat opposite the target quadrant (the quadrant where the platform was). Mice were allowed to swim in the maze for 60 s. The latency to find the platform and the swimming paths were recorded by a video camera and analysed by EthoVision software (Version 3.1, Noldus, The Netherlands).

2.4. Open field test

Mice were placed in an open arena (24.32 × 24.32 cm²) and detected by infrared photobeams apparatus (TRUE SCAN PHOTOB EAM LINC, COULBOURN) for 15 min. Two sensor frames, each consisting of a 32 × 32 photobeam array, were placed at 1 cm and 6 cm above the bottom of the cage and designed to detect movements in the horizontal and vertical planes.

2.5. Rotarod

Mice were placed on the rotarod (RT-01, SINGA, TAIWAN) and then sequentially started to rotate from 20 to 90 rpm with accelerating speed 10 rpm/10 s. Each speed rate was maintained for 5 s. The latency of each animal fall off the rotating rod was measured. Each animal was tested three times each experiment.
2.6. Measurement of Aβ levels

Frozen brain samples were used to measure Aβ levels. Hippocampi were homogenised in 5 M guanidine/5 mM Tris pH 8.0. The samples were further diluted as required for the assay with ice cold 0.25% casein blocking buffer that contained 0.5 M guanidine and protease inhibitor (Roche, Switzerland). Aβ levels were determined by enzyme-linked immunosorbent assay (ELISA) with a Human Amyloid β (1–x) and (1–42) Assay kits according to the manufacturer’s protocol (27729 and 27711, IBL, Japan).

2.7. Amyloid plaque staining and quantification

To detect the plaque burden, fixed brains from each group were examined histologically. Fixed brains were soaked in 30% sucrose, embedded in Optimal Cutting Temperature Compound (Sakura Tisexam) and sectioned with a cryostat microtome (Leica). Fixed brains were soaked in 30% sucrose, embedded in Optimal Cutting Temperature Compound (Sakura Tisexam) and sectioned with a cryostat microtome (Leica). Sections (Neuromics, Minneapolis, MN). Briefly, pieces of cortex were purchased from Neuromics Antibodies (Edina, MN). A single cell suspension was prepared from time-dependent pregnant rats 18-day-old foetuses obtained from time-dependent pregnant rats. Cells were treated with cell culture media supplemented with 2 mg/ml Pa-

2.8. Primary neuronal cultures

Primary rat basal forebrain neurons were cultured from 17- to 18-day-old foetuses obtained from time-dependent pregnant rats purchased from Neuromics Antibodies (Edina, MN). A single cell suspension was prepared following the manufacturer's instructions (Neuromics, Minneapolis, MN). Briefly, pieces of cortex were treated with cell culture media supplemented with 2 mg/ml Pa-

2.9. Aβ25–35 and crude caffeine extract treatment

The amyloid-β peptide 25–35 (Aβ25–35) was purchased from GenScript (Piscataway, NJ). Aβ25–35 was dissolved in filtered water and stored in 2 mM aliquots at −80 °C. On the day of the experiment, 20 μM of Aβ25–35 peptide was added to the appropriate wells, and the cells were cultured for 24 h. Cells were then treated with media containing 0, 0.5, 5, or 50 ng/ml of CC for 2 h. After the CC treatment, the media was removed, and the cells were washed once with fresh media that did not contain CC. Cultures were then treated with Aβ25–35 in culture media for 24 h. Cell culture reagents were purchased from Invitrogen (Carlsbad, CA), and foetal bovine serum was purchased from Atlanta Biologicals (Lawrenceville, GA).

2.10. Adenosine triphosphate (ATP) assay

After treatment with 20 μM Aβ25–35 and CC for 48 h, the bioluminescent ATP levels of the neurons were measured as an indicator of neuron viability. The cell lysis and ATP detection buffers were purchased from Promega (Wisconsin, WI).

2.11. Caspase-3 activity assay

After treatment with 20 μM Aβ25–35 and CC for 48 h, caspase-3 activity was measured with a fluorescently labelled caspase-3 kit. The caspase detection reagent was purchased from Caymen (Ann Arbor, MI).

2.12. Neuronal cell death assay

Neurons were cultured for 6 days and then treated with 20 μM Aβ25–35 for 24 h. Cells were treated with either media alone, media containing 50 ng/ml of CC or 45 ng/ml caffeine for 2 h. After caffeine or CC treatment, the media was removed, and the cells were washed once with fresh media that did not contain either type of caffeine. Cultures were replenished with Aβ25–35 in culture media and cultured for 24 h. After treatment, neurons were washed with PBS and fixed in 4% paraformaldehyde at room temperature for 20 min. The cells were washed with PBS three times for 5 min each and permeabilized with 0.2% Triton X-100 for 10 min. After incubation, Triton X-100 was removed, and the cells were blocked with 3% normal donkey serum for 60 min. Neurons were then stained with anti-vesicular acetylcholine transporter (VACHT, Millipore, Billerica, MA) and anti-active caspase-3 antibodies (Cell Signaling Technology, Danvers, MA), then observed under a fluorescence microscope. Multiple images of neurons were taken for each sample, and four fields of neurons were counted for each treatment condition.

2.13. Statistical analysis

Data were analysed by two-way and one-way analysis of variance (ANOVA) followed by a post hoc comparison test and unpaired t-test with PASW Statistics 18 (Chicago, IL) and GraphPad Prism (GraphPad Software Inc, La Jolla, CA). Results were expressed as the mean ± standard error of the mean (SEM), and p < 0.05 was considered statistically significant.

Table 1

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<tr>
<th>Body weight (g)</th>
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<th>APP-Cont</th>
<th>APP-CC</th>
<th>APP-Caff</th>
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<td>25.24 ± 1.14</td>
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<td>Final</td>
<td>31.64 ± 1.45</td>
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<tr>
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<table>
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<th>APP-Cont</th>
<th>APP-CC</th>
<th>APP-Caff</th>
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<td>5.65</td>
<td>6.10</td>
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</table>
3. Results

3.1. Crude caffeine improves learning and memory in a murine model of AD

To investigate whether CC and caffeine administration could influence spatial memory, APP transgenic mouse were fed either a control diet (APP-Cont), a CC-containing diet (APP-CC), or a caffeine-containing diet (APP-Caff) from 3 months of age until the time the mice were sacrificed. Their wild-type littermate controls (WT) were also fed with control diet at the same time. There were no significant differences between the groups in terms of food consumption per week, as mice in all the groups consumed approximately 35 g/week (Table 1). We estimated that each mouse consumed about 1.84 mg caffeine of CC per day or 60 mg/kg body weight. It is roughly equivalent to human daily consuming 4.86 mg/kg body weight of caffeine, or 291.60 mg of caffeine for 60 kg adult per day. This caffeine dose is less than 2 cups of regular coffee per day and quite in line with the average daily consumption of caffeine. This conversion is based on the body surface area normalisation method as previously reported (Reagan-Shaw, Nihal, & Ahmad, 2008). There were no differences in either average body weight or weight gain between the groups before and after different diet consumption (Table 1).

After 2 months of feeding, we performed the Morris water maze test to evaluate their spatial learning and memory. In the hidden platform test, control APP-Cont mice took a significantly longer time to find the platform than WT mice, suggesting that APP-Cont mice had impairments in spatial learning and memory. After 5 consecutive days of test, the APP-CC and APP-Caff mice had significantly better spatial memory acquisition than APP-Cont mice (Fig. 1A). Further, APP-CC mice found the platform quicker than APP-Caff mice, and a significant difference in the time to reach the platform was observed on the fifth day of testing (Fig. 1A). This result suggested that CC feeding was more effective at preventing spatial memory impairment than caffeine in APP transgenic mice.

We next tested whether 2 months CC and caffeine consumption could improve the retention of memory. To investigate memory retention, we performed a probe trial 24 h after the last day of hidden platform test. We removed the circular platform from the water maze, put the mice in the quadrant opposite to the target quadrant, and measured the time that the mice spent in the former platform-containing quadrant. Higher percent time spent in target quadrant indicates better memory retention. We found that APP-Cont mice spent less time in the correct quadrant than WT mice, suggesting memory retention impairment in APP transgenic mice (Fig. 1B). However, APP-CC mice showed significantly better memory retention than APP-Cont mice (Fig. 1B). APP-Caff mice had a trend of increased memory retention, but this difference was not significant. We did not see any significant differences in swimming velocity among the groups (data not shown). This indicated that the latency to find the platform and the duration in the target quadrant can be used as reliable measurements of spatial learning and memory in these mice. We also performed the Morris water maze on the WT mice feeding with CC or caffeine diet, and found no significant difference in their spatial learning and memory tasks (data not shown). In all, these results indicate that the consumption of CC may lead to better spatial learning and memory in APP transgenic mice.

As the differences in water maze performance may due to the change in motor activity and anxiety, we also monitored the performance of these mice in the open field and rotarod tests. In open field test, the total distance represents general locomotor activity for horizontal movement, and the percent distance in the centre region could be used as a putative measure for anxiety. Similar to previous open field study, all APP mice have higher total movement and lower percent centre movement than WT mice during 15 min testing (Cheng et al., 2007), but APP mice fed with different diets had no significant differences among groups (Fig. 1C and D). When using accelerating rotarod test to monitor motor coordination, APP-Caff mice stayed longer on the rotating rod than all other groups, suggesting the altered motor coordination in APP-Caff but not APP-CC mice. Therefore, the improvement in memory test of APP-CC mice is not due to the changes in motor activity and anxiety.

3.2. Crude caffeine suppresses Aβ levels and Aβ accumulation in AD mice

An increased number of cerebral plaques laden with Aβ peptide is one of the pathological hallmarks of AD. Aβ1–42 is more aggregation-prone and toxic than other Aβ species. To investigate whether CC consumption can modify the levels of Aβ in APP mice, we measured the amount of Aβ1–42 in the hippocampal lysates of AD mice with different diets. Two months of CC, but not caffeine, administration significantly reduced the levels of Aβ1–42 in APP mouse brains, suggesting that CC could delay Aβ1–42 deposition (Fig. 2A). There were no significant differences in the levels of total Aβ among the three groups (Fig. 2B). Thus, the ratio of Aβ1–42/total Aβ was lower in APP-CC mouse (Fig. 2C). Further, we quantified the number of plaques in the hippocampus with thioflavine-S staining. Intake of CC, but not caffeine, suppressed the number of amyloid plaques in the hippocampus (Fig. 2D and E). These results suggest that the beneficial effects of CC in the reduction of the memory impairment of the disease might be mediated by an Aβ-dependent mechanism.

3.3. Crude caffeine suppresses Aβ-induced neuronal cell death and caspase-3 activity

To investigate the neuroprotective effect of CC, we treated primary neurons with 20 µM Aβ25–35 for 24 h. The cells were then treated with media that contained 0, 0.5, 5, or 50 ng/ml CC for 2 h. After CC exposure, the media were removed, the cells were washed once with fresh media, and cultures were exposed to media that contained Aβ25–35 for another 24 h. The levels of Aβ accumulation in AD mouse were measured as an indicator of survival. Following this 48-h treatment with Aβ25–35, the level of Aβ accumulation in AD mouse was 46.8% of that found in control non-Aβ25–35-treated neurons. Interestingly, this decrease was limited by CC, as Aβ25–35 treated neurons exposed to 0.5, 5, and 50 ng/ml CC had Aβ levels of 57.1%, 68.3%, and 76.6%, respectively, of that found in control neurons (Fig. 3A). These results indicate that protective effect of CC against Aβ25–35-induced neuronal damage.

We next measured a different cell death indicator, caspase-3 activity. After 48-h of treatment with Aβ25–35, caspase-3 activity was increased up to 254% compared to control non-Aβ25–35 treated neurons. Aβ25–35-treated neurons exposed to 0.5, 5, and 50 ng/ml CC had caspase-3 activities of 189.7%, 174.4%, and 161.4%, respectively, of that found in control neurons (Fig. 3B). We further confirmed our finding by counting the caspase-3-positive neurons exposed to Aβ25–35 treated neurons exposed to 0.5, 5, and 50 ng/ml CC had ATP levels of 57.1%, 68.3%, and 76.6%, respectively, of that found in control neurons (Fig. 3A). These results indicate the protective effect of CC against Aβ25–35-induced caspase-3 activity.
4. Discussion

This study demonstrated that both CC- and caffeine-containing diet improved learning and memory in a mouse model of AD. Interestingly, CC had greater effects than caffeine on learning and memory performance in APP transgenic mice (Fig. 1). Our study confirmed previous studies that caffeine improves memory in a murine model of AD (Arendash et al., 2006, 2009). However, our study differed from the previous studies as we aimed to investigate the effects of CC and caffeine at the early stages of disease to investigate whether an early intervention could prevent the pathology and symptoms that occur later in the progression of the disease.

It has been demonstrated that caffeine intake enhances performance and increases neuromuscular coordination. Caffeine increases the concentration of serotonin in brain stem regions that project to spinal motor neurons and innervate skeletal muscle motor units (Walton, Kalmar, & Cafarelli, 2002). Therefore, we also measured the motor function of the animals after CC and caffeine consumption. In open field test, we did not see any differences in performance after Caffeine or CC consumption. Interestingly, in rotarod test, only caffeine, but not CC, showed enhanced performance in APP mice. Also, our animals did not show differences in swimming velocity during Morris water maze testing. These observations demonstrate that our memory testing was not confounded by an increase in motor function after CC consumption.

The disruption of Aβ homeostasis is one of the factors that contribute to the pathogenesis of AD. Changes in Aβ metabolism that lead to increased total levels of Aβ or the level of Aβ1–42 may result in Aβ deposition in the brain (Tanzi & Bertram, 2005). Our results showed that CC feeding reduced Aβ1–42 levels in the hippocampi of AD mice (Fig. 2A), which is in agreement with a previous study (Arendash et al., 2006). This result suggests that the by-products of decaffeination process might slow down the progression of the disease through an Aβ-dependent mechanism. The mechanism of how CC reduced the levels of Aβ remains unclear, but a recent study showed that caffeine prevented Aβ accumulation by reducing the
expression of APP and Aβ converting enzyme, an enzyme that cleaves APP into Aβ (Prasanthi et al., 2010). Similar to our findings, long-term caffeine administration was demonstrated to improve memory by reducing Aβ levels through the suppression of the Aβ-producing enzymes, β-, and γ-secretases (Arendash et al., 2006; Cao et al., 2011). These findings may at least in part explain the mechanism of how CC and pure caffeine alter Aβ levels in the brain.

Furthermore, our results showed that CC reduced Aβ1–42 levels and the number of amyloid plaques in the hippocampus (Fig. 2A and D). These results indicate that CC is more effective than pure caffeine at inducing Aβ clearance and/or suppressing Aβ accumulation. The high level of antioxidant activity in CC (Chu et al., 2012) may reduce Aβ aggregation by neutralising peroxynitrite, a reactive nitrogen species that adds a nitrate group to tyrosine 10 of Aβ and induces plaque formation, and/or other related oxidants (Kummer et al., 2011). Furthermore, the increased anti-inflammatory activities of CC (comparing to caffeine) (Chu et al., 2012) may allow microglia to maintain their phagocytic function and clean up small aggregates of Aβ. Inhibition of COX-2 and the prostaglandin E2 receptor have been shown to be correlated with the stimulation of phagocytosis by microglia and Aβ1–42 reduction (Lleo et al., 2004). However, further studies are needed to clarify the precise mechanism through which CC mediates its effects on Aβ clearance and/or formation.

A few molecular mechanisms may be involved in caffeine's protection against memory impairment. It is known that the blocking of the adenosine A2A receptor by caffeine protects neurons and synapses from Aβ toxicity (Canas et al., 2009; Cunha & Agostinho, 2010). In another study, it was shown that 10 mg/kg caffeine treatment for four consecutive days increases the performance of mice in the object recognition task (Costa et al., 2008). Long-term coffee consumption may be beneficial for the maintenance of memory because BDNF and TrkB protect against memory impairment and regulate neurogenesis in the hippocampus through an ERK-associated pathway (Nagahara et al., 2009). Long-term coffee treatment has also been shown to prevent sleep deprivation-induced impairments in hippocampal-dependent short term memory and the early phase of long-term potentiation (LTP). These protective effects were correlated with an increase in calcium-calmodulin dependent kinase II, a serine/threonine-specific protein kinase known to be the regulator of short-term memory and LTP, after caffeine treatment (Alhaider, Aleisa, Tran, & Alkadhi, 2010). These mechanisms may explain our results that indicate CC and caffeine improve cognition and memory retention (Fig. 1). For a balanced evaluation of the bioactivities of caffeine, the side effects of caffeine should be also noted. It is well known that high level of caffeine consumption (>500 mg/day) by humans can lead to headaches, restlessness or anxiety (Hagen, Thoresen, Stovner, & Zwart, 2009; Kaplan et al., 1997). To achieve the same dose for caffeine intake in this study, a 60 kg adult should take 291.60 mg caffeine per day (Reagan-Shaw et al., 2008). This dose is less than 500 mg/day, but may still have some potential side effect depending on the health condition.

Progressive neuronal loss in the hippocampus and cortex is one of the characteristics of AD that cause deficits in learning and
memory (Mattson, 2004). Apoptosis and increased activated caspase-3 immunoreactivity have been observed in neurons in AD brains (Friedlander, 2003). The results in Fig. 3 imply that CC and caffeine can rescue cholinergic neurons from Aβ toxicity and suppress Aβ-induced caspase-3 activation. Interestingly, although the CC treatment showed greater effects in the Morris water maze test (Fig. 1A), there were no significant differences between the CC and caffeine treatments in the protection of neurons against Aβ toxicity (Fig. 3C and D). This may be explained by our previous report that CC is more effective than caffeine at reducing the level of the pro-inflammatory protein COX-2 (Chu et al., 2012). We speculate that both the antioxidant and anti-inflammatory activities of CC function in the animal model in which neurons and glia coexist, but only the antioxidant activities have effects in the neuronal cell cultures with very little glia exist (cultures are over 95% neurons). If this is indeed the case, it may explain the differential effects of CC and caffeine in our in vitro experiments. This observation suggests that both the anti-inflammatory and antioxidant characteristics of CC are critical in the protection against a multifactorial neurodegenerative disease such as AD.

Crude caffeine is produced in huge amounts in the coffee decaffeination process. After caffeine purification, the non-caffeine residues are discarded as waste. Here, we show that CC has beneficial effects in the brain, as it improved memory function and reduced hippocampal Aβ1–42 levels in a mouse model of AD. CC also inhibits Aβ25–35 induced cell death. Our findings suggest that crude caffeine could contain prophylactic agents and identification of the active component could be economically attractive.

Conflict of interest

Y. Chu and R. Black are employees of Kraft Foods Global Brands LLC.

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