Caffeine blocks cholesterol-enriched diet induced AD-like pathology in rabbits

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Abstract

The pathogenesis of sporadic AD is believed to result from complex interactions between nutritional, environmental, epigenetic and genetic factors. Among those factors, elevated plasma cholesterol, independent of APOE genotypes, is strongly linked to the pathogenesis of sporadic AD, whereas chronic ingestion of caffeine is protective against sporadic AD. Others and we have shown that rabbits-fed cholesterol-enriched diet exhibit early AD-like pathological features including blood-brain barrier (BBB) leakage and endolysosome dysfunction, as well as, AD-like pathological hallmarks including synaptic disruption, increased intraneuronal and brain deposition of amyloid beta (Aβ), and tau pathology. Further, we found that caffeine protects against the cholesterol-induced increases in BBB permeability. Here, we determined the extent to which caffeine affects cholesterol-enriched diet-induced increases in other pathological features of AD. We demonstrated that caffeine administration at low and moderate doses did not affect plasma levels of cholesterol, but did block significantly cholesterol-enriched diet-induced increases in brain levels of apoB and accumulation of apoB in neuronal endolysosomes. Furthermore, we demonstrated that caffeine administration at the two doses blocked cholesterol-enriched diet-induced abnormal accumulation of synaptophysin, Aβ, and phosphorylated tau in endolysosomes. Our findings suggest that caffeine exerts its protective effects against the development of sporadic AD-like pathological features, in part, by preventing the entrance of LDL cholesterol into brain parenchyma, the accumulation of LDL-cholesterol in neuronal endolysosomes, and structural and functional changes to endolysosomes.

Introduction

Alzheimer's disease (AD), the most common neurodegenerative disorder of old age, is characterized clinically by a progressive decline in cognitive function, and pathologically by loss of synaptic integrity and neurons, amyloid plaques composed of amyloid beta (Aβ) protein, and neuronal tangles composed of hyperphosphorylated tau [1,2]. Although mutations in specific genes, including AβPP, PSEN1, and PSEN2, lead to rare early-onset familial forms of AD, the vast majority of AD cases are late in onset and sporadic in nature. The pathogenesis of sporadic AD is much more complex than that of familial AD and sporadic AD is believed to result from complex interactions between nutritional, environmental, epigenetic and genetic factors [3]. Of those factors, altered cholesterol homeostasis may have the strongest links to the pathogenesis of late-onset sporadic AD.

Evidence for the involvement of cholesterol dyshomeostasis in AD pathogenesis comes principally from the following findings. First, the ε4 allele of the APOE gene, which encodes the major cholesterol carrier protein in brain apolipoprotein E (apoE), is the strongest genetic risk factor yet identified for sporadic AD. Second, apoE4 is associated with elevated levels of plasma cholesterol [4,5]. Third, elevated levels of plasma cholesterol, independent of APOE genotypes, has been linked to the pathogenesis of sporadic AD [6-10], as supported by epidemiology studies [6,9] as well as experimental studies conducted in AβPP transgenic mice [11,12], in guinea pigs [13], in rabbits [14], and in rats [15].

Rabbits fed a high cholesterol diet are considered to be a good model for sporadic AD. We have shown that the cholesterol-fed rabbits develop pathological hallmarks of AD including disrupted synaptic integrity, brain deposition of Aβ, and tau pathology [7]. In addition, we have reported that cholesterol-fed rabbits exhibit very early pathological features found in people with AD including increased blood-brain barrier (BBB) leakage [7,16,17] and disturbed neuronal endolysosome structure and function [18]. Furthermore, using primary cultured neurons we demonstrated that LDL cholesterol, the major lipoprotein particle responsible for cholesterol transport in the blood, promoted AβPP internalization, enhanced BACE-1 activity, and increased amyloidogenic processing of AβPP in endolysosomes [8]. Collectively, our findings suggest that elevated levels of LDL cholesterol can enter brain parenchyma via a leaky BBB whereby the LDL cholesterol enters into endolysosomes via receptor-mediated endocytosis and disturbs the structure and function of neuronal endolysosomes thus promoting the development of AD-like pathology.

Human epidemiological and animal experimentation studies as well as studies conducted in cultured cell models have shown that caffeine protects against the onset and severity of AD [19-28]. Additionally, it has been shown that it can reverse behavioral and pathological features of AD [19,21,22,24,25,29-33]. Importantly, these protective effects of caffeine are observed using pharmacologically relevant doses [28,34]. Previously, using the cholesterol-fed rabbit model of sporadic AD, we reported that chronic ingestion of caffeine protects against cholesterol-

induced increases in BBB leakage, oxidative stress, brain deposition of Aβ, and tau phosphorylation [16, 35,36]. The present studies were aimed to determine the effects of pharmacologically relevant very low and higher doses of caffeine on AD-like pathology using the cholesterol-fed rabbit model for sporadic AD with a specific focus on determining the extent to which endolysosomes are involved.

Material and methods

Animals and treatments: New Zealand White male rabbits (1.5–2 years of age and weighing 3 to 4 kg; Charles River Laboratories International, Wilmington, MA, USA) were assigned randomly to the following four groups (n=4 each); rabbits fed normal chow, rabbits fed a 2% cholesterol-enriched diet (Harlan Teklad Global Diets, Madison, WI, USA), rabbits fed a 2% cholesterol-enriched diet + 0.5 mg of caffeine/day, and rabbits fed a 2% cholesterol-enriched diet + 30 mg of caffeine/day. All rabbits were kept on their respective diet and caffeine treatment for 12 weeks after which time the rabbits were euthanized. Caffeine was prepared fresh daily and was administered in the drinking water. The very low (0.5 mg/day) and higher (30 mg/day) doses of caffeine were chosen based on findings that the basal metabolic rate in animals is proportional to the 3/4 power of their body mass; the metabolic rate for rabbit is about twice that of a human. Thus, rabbits ingesting 0.5 mg caffeine per day is roughly equivalent to a 70 Kg person ingesting 18 mg of caffeine; a dose well below that contained in a single serving of virtually all caffeinated beverages. At necropsy, animals were perfused with Dulbecco's phosphate-buffered saline at 37°C, and olfactory bulbs were dissected, frozen on a liquid nitrogen-cooled surface and stored at – 80°C until taken for experimentation; one olfactory bulb from each animal was taken for immunostaining and the other for immunoblotting. All animal procedures were carried out in accordance with the U.S. Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of North Dakota.

Plasma cholesterol levels: Total levels of plasma cholesterol in rabbits were measured in blood collected from an ear vein after overnight fasting immediately before euthanasia. The measurements were carried out using a Flex reagent cartridge and the Dimension Clinical Chemistry System (Dade Behring, Inc.).

Immunohistochemistry: Snap-frozen olfactory bulbs were embedded in Tissue-Tek Optimum Cutting Temperature (OCT) compound in cryomolds. Horizontal cryostat sections (thickness 14 μm) were mounted on Superfrost Plus slides (Fisher). For immunofluorescent staining studies, sections were air-dried at room temperature for 40 minutes, fixed in ice-cold acetone for 10 minutes, air-dried for 30 minutes, and washed 3-times with PBS. Sections were then blocked with 5% donkey serum and incubated overnight at 4oC with primary antibodies targeting synaptophysin (1:1000, mouse monocolonal, Sigma), EEA1 (1:500, goat polycolonal, Santa Cruz), LAMP2 (1:500, goat polycolonal, Santa Cruz), apolipoprotein B (1:500, mouse monoclonal, Santa Cruz), Aβ/APP (6E10, 1:500, mouse monoclonal, Signet), and phospho-tau (AT8, 1:500, Pierce). After 3 washes with PBS, sections were incubated with corresponding fluorescence-conjugated secondary antibodies including Alexa 546-conjugated donkey anti-mouse antibodies (Invitrogen), Alexa 488-conjugated donkey anti-mouse antibodies (Invitrogen), and Alexa 546-conjugated donkey anti-goat antibodies (Invitrogen). Neurons were identified with fluorescence-based NeuroTrace Nissl stain (1:1000, Invitrogen). Sections were examined using an Olympus

confocal microscope. Controls for specificity included staining olfactory bulb sections with primary antibodies without fluorescence-conjugated secondary antibodies (background controls), and staining olfactory bulb sections with only secondary antibodies – these controls eliminated auto-fluorescence in each channel and bleed-through (crossover) between channels. Fluorescence intensity was quantified using Image J software.

Immunoblotting: Olfactory bulbs were gently homogenized using a teflon homogenizer (Thomas) in 10 volumes of ice-cold suspension buffer (20 mM HEPES-KOH (pH 7.5), 250 mM sucrose, 10 mM KCl, 1.5 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.1 mM PMSF, 2 mg/ml aprotinin, 10 mg/ml leupeptin, 5 mg/ml pepstatin, and 12.5 mg/ml of N-acetyl-Leu-Leu-Norleu-Al). Protein concentrations were determined with a Bradford protein assay (Bio-Rad). Proteins (10 μg) were separated by SDS-PAGE (12% gel), and following transfer to polyvinylidene difluoride membranes (Millipore) they were incubated overnight at 4°C with antibodies against synaptophysin (1:1000), N-terminal AβPP (1: 1000), C-terminal AβPP (1: 500, Sigma), apolipoprotein B (1:1000, Santa Cruz) or AT8 (1:1000, Pierce): β-actin (1: 10000, Abcam) was used as a gel loading control. The blots were developed with enhanced chemiluminescence, and bands were visualized and analyzed by LabWorks 4.5 software on a UVP Bioimaging System (Upland). Quantification of results was performed by densitometry and the results were analyzed as total integrated densitometric volume values (arbitrary units).

Statistical analysis: All data were expressed as means and SEMs. Statistical significance was analyzed with one-way ANOVA plus a Tukey post-hoc test. $P < 0.05$ was considered to be statistically significant.

Results

Caffeine prevented cholesterol-enriched diet-induced neuronal accumulation of apoB: We published previously that cholesterol-enriched diet disrupts the BBB [16]. Thus, elevated levels of plasma cholesterol may enter brain parenchyma and may promote neuronal uptake via receptor-mediated endocytosis. Indeed, we have shown that cholesterol-enriched diet increased neuronal endolysosome accumulation of LDL cholesterol derived from the systemic circulation as evidenced by findings that apoB, the exclusive apolipoprotein in LDL particles that is not normally found in brain [37], is accumulated in neuronal endolysosomes from rabbits fed a cholesterol-enriched diet. In addition, we found that the chronic injection of caffeine prevented cholesterol-enriched diet-induced disruption of the BBB [16]. Here, we determined the extent to which caffeine affected plasma levels of cholesterol and neuronal accumulation of cholesterol of peripheral origin using apoB as a marker. We found that the cholesterol-enriched diet markedly increased plasma cholesterol levels from 64 ± 7.4 to 658 ± 101.5 mg/dl, and that caffeine administration at the two different doses did not affect significantly plasma cholesterol levels in either control rabbits (84 ± 5.0 mg/dl for 0.5 mg/day and 88 ± 12.6 mg/dl for 30 mg/day) or cholesterol-fed rabbits (594 ± 124.6 mg/dl for 0.5 mg/day, and 743 ± 92.4 mg/dl for 30 mg/day). However, caffeine administration did affect cholesterol-enriched diet-induced brain accumulation of cholesterol of peripheral origin. Immunohistochemically, we demonstrated in olfactory bulb glomeruli that the cholesterol-enriched diet increased markedly neuronal accumulation of apoB, which was blocked by caffeine ingestion at both low (0.5 mg/day) and higher (30 mg/day) doses (Figure 1A). Furthermore, we demonstrated in rabbits fed a cholesterol-enriched diet that apoB was accumulated in EEA-
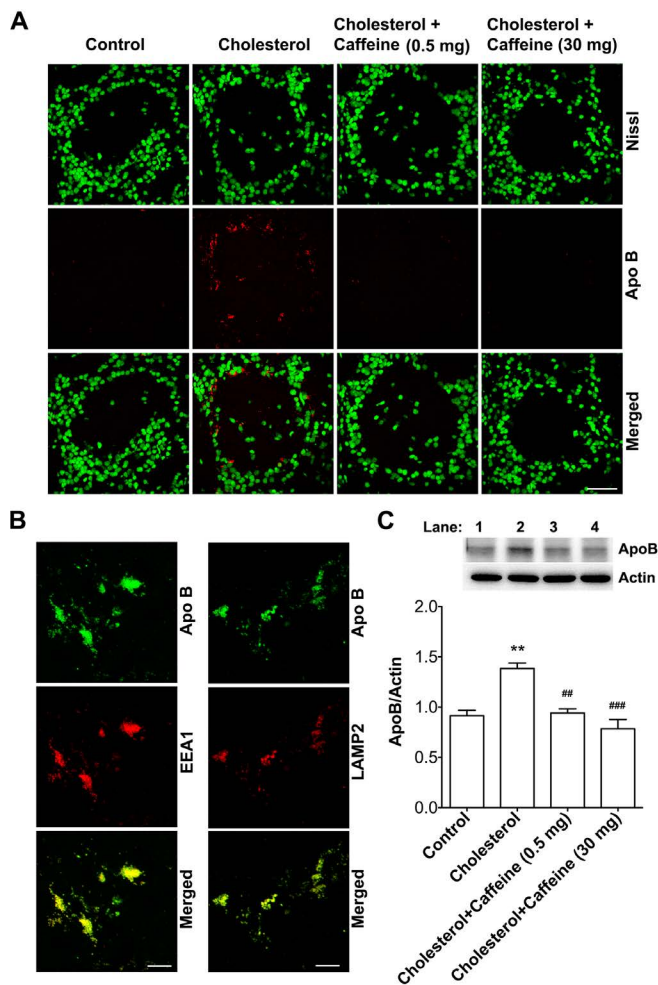
Figure 1. Caffeine prevented cholesterol-enriched diet-induced neuronal accumulation of apoB.

(A) Cholesterol-enriched diet markedly increased immunoreactivity of apoB (red) in neurons (Nissl, green) in olfactory bulb glomeruli, and such an effect was blocked by caffeine at two different doses (0.5 or 30 mg/day). Bar=50 μm. (B) In olfactory bulbs from cholesterol-fed rabbits, immunoreactivity of apoB co-distributed with endosomes (EEA1) and lysosomes (LAMP2) in the glomerular layer. Bar=20 μm. (C) Cholesterol-enriched diet increased significantly protein levels of apoB in olfactory bulbs, and such an effect was blocked by caffeine at two different doses (n=4, *p<0.05). Lane 1, Control; Lane 2, Cholesterol; Lane 3, Cholesterol + Caffeine 0.5 mg; Lane 4, Cholesterol + Caffeine 30 mg.

1 positive endosomes and LAMP2 positive lysosomes (Figure 1B). Using immunoblotting methods, we quantified the protein levels of apoB in olfactory bulbs and found that the cholesterol-enriched diet increased significantly protein levels of apoB in olfactory bulbs and that this increase was blocked by caffeine ingestion at low (0.5 mg/day) and higher (30 mg/day) doses (Figure 1C). Consistent with our findings that caffeine protects against BBB leakage, these observations indicate that caffeine prevents accumulation in neurons and in brain of cholesterol derived from systemic circulation sources.

Caffeine prevented cholesterol-enriched diet-induced synaptic loss: Synaptic loss is a pathological hallmark of AD. Here, we determined the extent to which caffeine affected synaptic integrity. Consistent with our previous findings, we demonstrated that cholesterol-enriched diet changed markedly the distribution of immunoreactive synaptophysin in glomeruli of olfactory bulb. In control rabbits, the distribution of synaptophysin immunoreactivity was fairly evenly distributed within

individual glomeruli. In cholesterol-fed rabbits, the synaptophysin immunoreactivity was markedly decreased in the center of glomeruli. In addition, intense granule-shaped immunoreactivity was observed very close to the cell body of periglomerular cells in cholesterol-fed rabbits (Figure 2A). These distribution changes were almost completely blocked with low (0.5 mg/day) and higher (30 mg/day) doses of caffeine (Figure 2A). In olfactory bulbs from rabbits fed a cholesterol-enriched diet, granule-shaped synaptophysin immunoreactivity was co-distributed with EEA1 and with LAMP 2 (Figure 2B). Furthermore, caffeine at the two different doses prevented significantly cholesterol-enriched diet-induced decreases in protein levels of synpatophysin.

Caffeine prevented cholesterol-enriched diet-induced brain deposition of Aβ: We reported previously that rabbits fed a cholesterol-enriched diet had increased brain deposition of Aβ, a
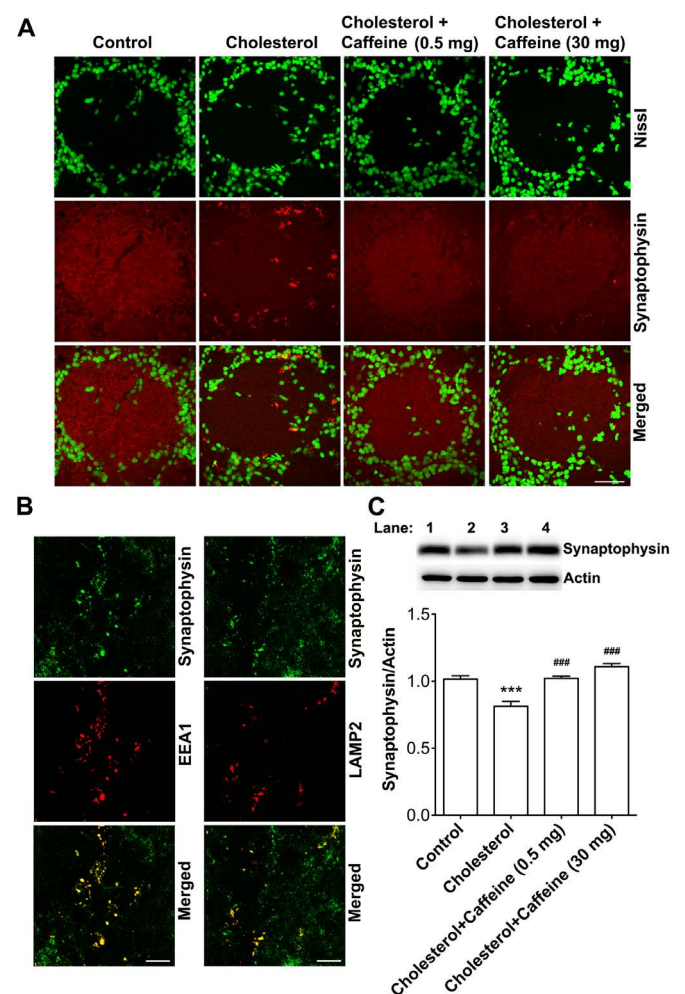

Figure 2. Caffeine prevented cholesterol-enriched diet-induced synaptic loss.

(A) Cholesterol-enriched diet changed markedly the distribution of synaptophysin in glomeruli of olfactory bulbs; the distribution pattern of synaptophysin immunoreactivity changed from an even distribution within individual glomeruli from control rabbits to decreased immunoreactivity in the center of glomeruli from cholesterol-fed rabbits, and intense granule-shaped immunoreactivity was observed very close to the cell body of periglomerular cells in cholesterol-fed rabbits, and such effects were blocked by caffeine at two different doses (0.5 or 30 mg/day). Bar=50 μm. (B) In olfactory bulbs from cholesterol-fed rabbits, immunoreactivity of synaptophysin co-distributed with endosomes (EEA1) and lysosomes (LAMP2) in glomerular layer. Bar=20 μm. (C) Caffeine at two different doses prevented significantly cholesterol-enriched diet-induced decreases in protein levels of synaptophysin (n=4, *p<0.05). Lane 1, Control; Lane 2, Cholesterol; Lane 3, Cholesterol + Caffeine 0.5 mg; Lane 4, Cholesterol + Caffeine 30 mg.

pathological hallmark of AD [7, 38]. Consistent with those findings, we demonstrated here that the cholesterol diet increased markedly 6E10 positive immunopositive staining of Aβ/AβPP in olfactory bulbs (Figure 3A) and that these increases were blocked by ingestion of low (0.5 mg/day) and higher (30 mg/day) doses of caffeine (Figure 3A). Furthermore, intracellular Aβ accumulated in EEA1 positive endosomes and LAMP2 positive lysosomes (Figure 3B). Using immunoblotting methods, we demonstrated that the cholesterol-enriched diet promoted amyloidogenic processing of AβPP (Figure 3C). Cholesterol-enriched diet did not affect significantly protein levels of full-length AβPP or C83 fragment, the product of the non-amyloidogenic β-secretase pathway. However, cholesterol-enriched diet increased significantly protein levels of C99 fragment, the cleavage product of AβPP catalyzed by β-site AβPP cleavage enzyme (BACE-

1). Furthermore, the cholesterol-enriched diet increased significantly protein levels of BACE1, the rate-limiting enzyme in amyloidogenic processing of AβPP. Moreover, caffeine ingestion at low (0.5 mg/day) and higer (30 mg/day) doses blocked cholesterol-enriched diet-induced increases in protein levels of C93 fragment of AβPP and BACE-1 (Figure 3C).

Caffeine prevented cholesterol-enriched diet-induced tau phosphorylation

Neurofibrillary tangles composed of phosphorylated tau are another pathological hallmark of AD and we showed previously that cholesterol-enriched diet increased tau phosphorylation in neurons [7,38]. Here, we determined the extent to which caffeine
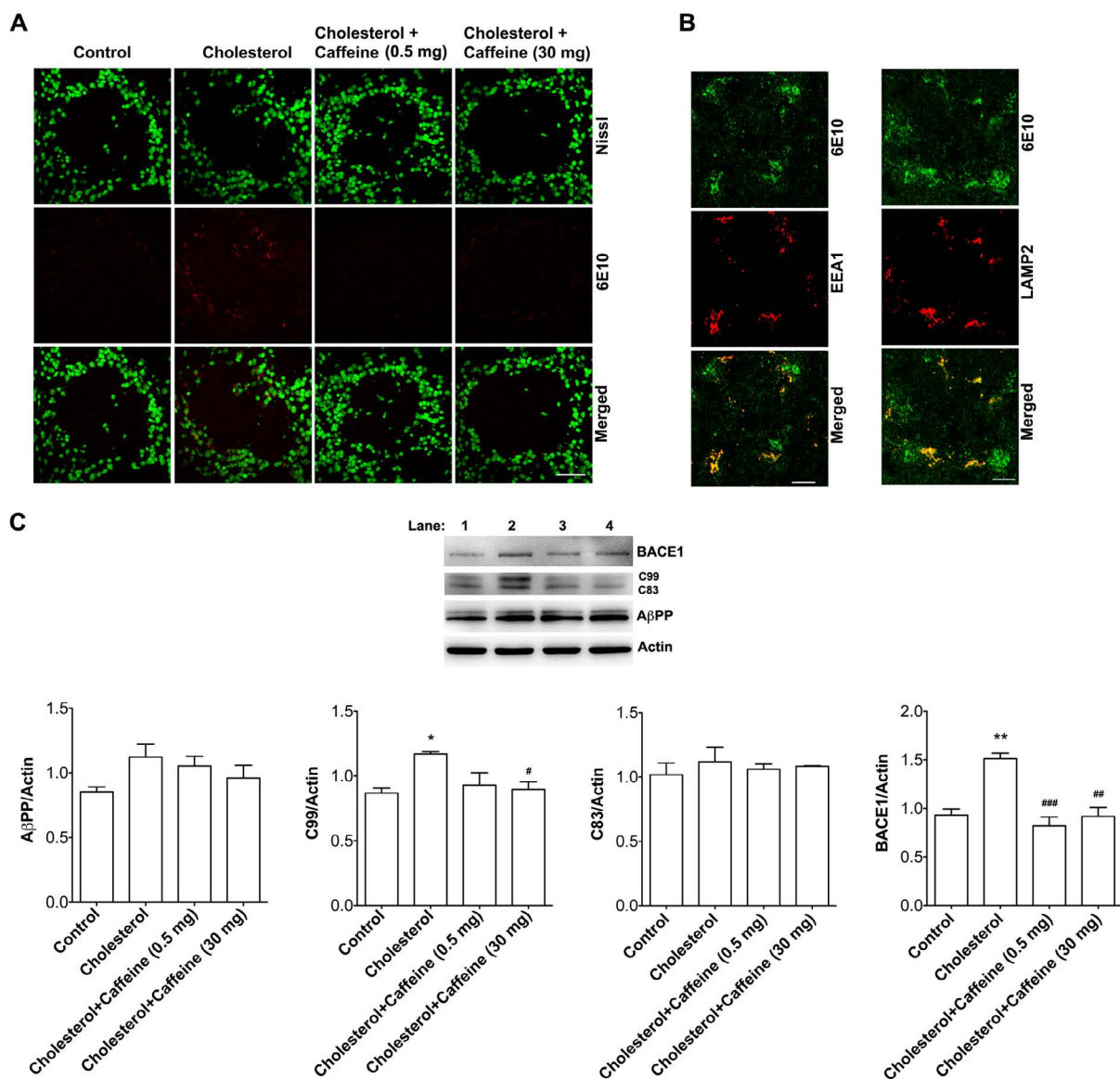

Figure 3. Caffeine prevented cholesterol-enriched diet-induced brain deposition of Aβ/APP.
(A) Cholesterol-enriched diet increased markedly 6E10 positive immunopositive staining of Aβ/AβPP in olfactory bulbs, and such effects were blocked by caffeine at two different doses (0.5 or 30 mg/day). Bar=50 μm. (B) In olfactory bulbs from cholesterol-fed rabbits, intracellular Aβ/AβPPimmunoreactivity co-distributed with EEA1 positive endosomes and LAMP2 positive lysosomes. Bar=20 μm. (C) Cholesterol-enriched diet did not affect significantly protein levels of full-length AβPP or C83 fragment, but increased significantly protein levels of C99 fragment of AβPP and BACE1, and such effects were blocked by caffeine at two different doses (n=4, *p<0.05). Lane 1, Control; Lane 2, Cholesterol; Lane 3, Cholesterol + Caffeine 0.5 mg; Lane 4, Cholesterol + Caffeine 30 mg.

affects tau phosphorylation in cholesterol-fed rabbits. Consistent with our previous findings, we demonsrated immunohistochemically that cholesterol-enriched diet increased neuronal deposition of phosphorylated tau (Figure 4A) and that these increases were blocked by ingestion of low (0.5 mg/day) or higer (30 mg/day) doses of caffeine (Figure 4A). In addition, we demonstrated in olfactory bulbs from cholesterol-fed rabbits increased accumulation of phosphorylated tau in EEA1 positive endosomes and LAMP2 positive lysosomes (Figure 4B). Furthermore, we found that caffeine ingestion at low (0.5 mg/day) and higer (30 mg/day) doses blocked cholesterol-enriched diet-induced increases in tau phosphorylation (Figure 4C).

Discussion

Consistent with findings from epidemiological and experimental studies that caffeine is protective against AD [29,34,39], we demonstrated here that chronic ingestion of caffeine at even very low doses exerts protective effects against the development of pathological

Figure 4. Caffeine prevented cholesterol-enriched diet-induced tau phosphorylation.
(A) Cholesterol-enriched diet increased markedly AT8 positive immunopositive staining of phosphorylated tau in olfactory bulbs and such effects were blocked by caffeine at two different doses (0.5 or 30 mg/day). Bar=50 μm. (B) In olfactory bulbs from cholesterol-fed rabbits, immunoreactivity of AT8 co-distributed with EEA1 positive endosomes and LAMP2 positive lysosomes. Bar=20 μm. (C) Cholesterol-enriched diet increased significantly protein levels of phosphorylated tau in olfactory bulbs, and such an effect was blocked by caffeine at two different doses (n=4, *p<0.05). Lane 1, Control; Lane 2, Cholesterol; Lane 3, Cholesterol + Caffeine 0.5 mg; Lane 4, Cholesterol + Caffeine 30 mg.

hallmarks of AD, including synaptic loss, brain deposition of Aβ, and tau pathology, in a cholesterol-fed rabbit model of sporadic AD.

The pathogenesis of sporadic AD is believed to result from complex interactions between nutritional, environmental, epigenetic and genetic factors [3]. Among those factors, elevated levels of circulating LDL cholesterol, independent of APOE genotypes, is strongly linked to the pathogenesis of sporadic AD [6-10]. Of mechanistic importance, we have shown that elevated levels of LDL cholesterol, the essential lipoprotein transporting circulating cholesterol in blood, (1) induces blood-brain barrier (BBB) leakage [7,16] - an early pathological feature of sporadic AD that precedes brain deposition of Aβ [17], (2) disturbs neuronal endolysosome structure and function - another early pathological features of sporadic AD [18], and (3) promotes the development of pathological hallmarks of AD including disrupted synaptic integrity, brain deposition of Aβ, and tau pathology [7]. Furthermore, we have demonstrated in cultured neurons that treatment with LDL cholesterol increased cholesterol accumulation in endolysosomes, disturbed the structure and function of endolysosomes, promoted amyloidogenic processing of AβPP, increased levels of phosphorylated tau, and decreased levels of the presynaptic protein synaptophysin [8].

Olfactory dysfunction has been deemed to be the earliest symptom of AD [40,41]. Deficits in ordor identification have been shown to increase the likelihood of subsequent cognitive decline, especially in patients carring one or two copies of the β4 allele of ApoE [40,42,43], the major genetic risk factor of sporadic AD. Thus, altered cholesterol homeotasis could contribute to the development of olfactory dysfunction in sporadic AD. Pathologically, oxidative damage, the presence of Aβ plaque, and accumulation of phosphorylated tau as neurofilrillary tangles all have been documented in olfactory epithelium and olfactory bulbs of AD patients [44-51]. Previously we showed in olfactory bulb from cholesterol-fed rabbits that elevated levels of circulating cholesterol disrupted the blood-brain barrier [16], increased accumulations of cholesterol in endolysosomes of neurons in olfactory bulb originated from peripheral sources, and contributed to the development of AD-like pathology including synaptic loss, elevated Aβ production, and increased tau phosphorylation [7].

Consistent with our previous findings, here we demonstrated in olfactory bulbs that cholesterol-enriched diet increased brain levels of apoB containing LDL cholesterol and promoted the accumulation of apoB, synpatophysin, Aβ, and phosphorylated-tau in endolysosomes. Collectively, our findings suggest that elevated levels of LDL cholesterol, when it enters brain parenchyma via a leaky BBB, are internalized by neurons via receptor-mediated endocytosis. Because LDL cholesterol is not normally present in brain and is foreign to neurons, the accumulation of LDL cholesterol in neuronal endolysosomes may induce a "traffic jam" in intracellular cholesterol transport, thereby disturbing endolysosome structure and function. Such LDL cholesterol-induced endolysosome dysfunction could contribute directly to the development of pathological hallmarks of AD including brain deposition of Aβ, synaptic loss, and tau pathology [8]. In support, (1) endolysosomes are the major site, where Aβ is produced [52,53], and endolysosome dysfunction leads to brain deposition of Aβ [17]; (2) Endolysosomes are responsible for synaptic protein recycling [54-56], and endolysosome dysfunction leads to disruption of synaptic integrity in brain [57-61]; (3) Tau can be degraded by cathepsin D in autophagosomes-lysosomes [62-65] and increased levels of endolysosome cholesterol, as occurs in Niemann-Pick type C disease, leads to lysosome dysfunction and tau-pathology [66-71].
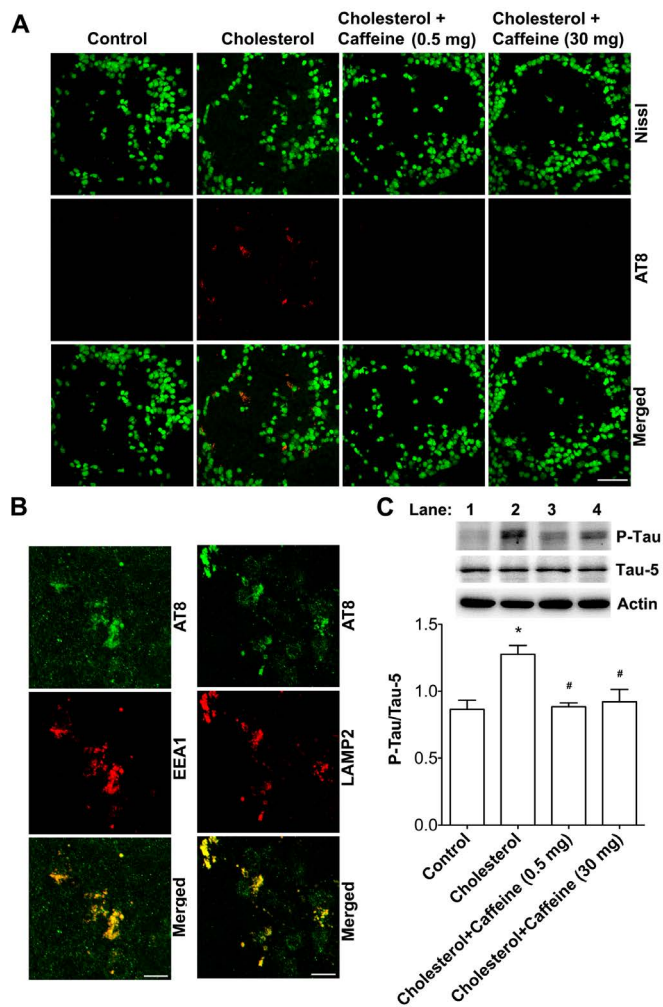
Substantial evidence from human epidemiological studies and from experimental studies conducted in animals and cultured cell models indicate that caffeine, when ingested chronically, can decrease Aβ levels, protect against the onset and severity of AD, and in some cases it can reverse behavioral and pathological features of AD [19,21,24,29]. Less clear, however, are the mechanisms by which caffeine exhibits these protective features. Previously, we have shown, in the cholesterol-fed rabbit model of sporadic AD, that chronic ingestion of caffeine protects against the disruption of the BBB [16]. As such caffeine may prevent the entrance of the foreign LDL cholesterol into brain parenchyma, thus protecting against subsequent LDL cholesterol-induced endolysosome dysfunction as well as the development of pathological hallmarks of AD. In support, we demonstrated that caffeine ingestion at two different doses did not affect plasma levels of cholesterol in either control rabbits or cholesterol-fed rabbits, but blocked cholesterol-enriched diet-induced elevation of brain levels of apoB, the key apolipoprotein of LDL particles. Importantly, we demonstrated that chronic ingestion of caffeine prevented cholesterol-enriched diet induced synaptic disruption, amyloidogenic processing of AβPP, and tau-pathology. In addition to its protective effects on the BBB, we have shown in neurons that caffeine inhibits LDL cholesterol internalization. As such caffeine could prevent abnormal accumulation of LDL cholesterol in neuronal endolysosomes, thus preventing LDL cholesterol-induced endolysosome dysfunction and subsequent development of AD-like pathology. Indeed, we demonstrated that caffeine blocked cholesterol-enriched diet-induced abnormal accumulation of synatophysin, Aβ, and phosphorylated tau in endolysosomes.

Together, our findings suggest that caffeine exerts its protective effects against the development of sporadic AD, in part, by preventing against entrance of LDL cholesterol into brain parenchyma and the accumulation of LDL-cholesterol in neuronal endolysosomes. Further elucidation of the involved mechanism may provide insight into the pathogenesis of sporadic AD and may lead to new effective therapeutic strategies against this devastating neurodegenerative disease.

Acknowledgment

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