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Phytochemicals from *Achillea fragrantissima* are Modulators of A β PP Metabolism

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Abstract

Plant derivatives offer a novel and natural source of therapeutics. The desert plant *Achillea fragrantissima* (Forssk) Sch. Bip (*Af*) is characterized by protective antioxidative and anti-inflammatory properties. Here, we examined the effect of two *Af*-derived phytochemicals on learning and memory, amyloid- β protein precursor (A β PP) metabolism, and tau phosphorylation in the familial Alzheimer's disease-linked APPswe/PS1 E9 mouse model. We observed that mice that were injected with the phytochemicals showed a trend of improvement, albeit statistically insignificant, in the Novel Object Recognition task. However, we did not observe improvement in contextual fear conditioning, suggesting that the benefits of treatment may be either indirect or task-specific. In addition, we observed an increase in the full-length form of A β PP in the brains of mice treated with *Af*-derived phytochemicals. Interestingly, both *in vivo* and *in vitro*, there was no change in levels of soluble A β , oligomeric A β , or the carboxyl terminus fragments of A β PP (APP-CTFs), suggesting that the increase in full length A β PP does not exacerbate A β PP pathology, but may stabilize the full-length form of the molecule. Together, our data suggest that phytochemicals present in *Af* may have a modest positive impact on the progression of Alzheimer's disease.

Keywords

Achillolide A; Alzheimer's disease; amyloid- β protein precursor; flavonoids; sesquiterpene lactones; 3, 5, 4'-trihydroxy-6, 7, 3'-trimethoxyflavone

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive loss of memory and cognitive dysfunction. Loss of neurons in the entorhinal cortex and hippocampus takes place in the disease, but the mechanism underlying neuronal vulnerability in these brain areas is not fully understood. Rare, familial forms (FAD), are

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caused by mutations in amyloid- β protein precursor (A β PP) and presenilin-1 (PS1). Expression of these mutant forms induces the amyloidogenic processing of A β PP, leading to the accumulation of oligomeric A β and its deposition in the form of amyloid plaques in the brain. The other pathological hallmark of the disease is hyperphosphorylation of tau, leading to neurofibrillary tangles. Oxidative stress and neuroinflammation contribute to the development of AD [1–3], and antioxidant and anti-inflammatory strategies appear to be an encouraging therapeutic approach [4–6]. Nevertheless, to date, there is no therapy for AD.

Plant derivatives offer a novel source of therapeutics. An infusion of the dry or fresh flowers of *Achillea fragrantissima* (*Af*) has long been known to have therapeutic effects [7–9]. We have previously reported that the extract of *Af* has anti-inflammatory [10] and anti-oxidant properties [11]. The sesquiterpene lactone achilolide A (AcA) and the 3,5,4'-trihydroxy-6,7,3'-trimethoxyflavone (TTF) are two bioactive compounds extracted from *Af*. We have shown that AcA is able to reduce microglial activation and to protect astrocytes from H₂O₂-induced cell death and oxidative stress *in vitro* [12, 13]. We have also shown that TTF protects astrocytes against oxidative stress, and N2a cells against A β -induced toxicity [14], via interference with cell signaling and by reducing the levels of intracellular reactive oxygen species [15]. Both compounds protected differentiated neuroblastoma N2a cells from glutamate toxicity [16].

Here, we investigated whether AcA or TTF can ameliorate learning and memory impairments and pathology in the FAD-linked mouse model, APP^{swe}/PS1^{E9}. We observed a trend of improvement, albeit statistically insignificant, in the performance of mice in the Novel Object Recognition task following treatment with *Af* phytochemicals, but not in contextual fear conditioning. Interestingly, we observed enhanced levels of full length A β PP but no increase in the level of its carboxyl terminus fragments (A β PP-CTFs), total soluble A β , or the amyloidogenic oligomeric A β . This may suggest that both AcA and TTF are modulators of A β PP metabolism. Our observations may implicate that AcA and TTF stabilize full length A β PP but do not enhance amyloidogenic processing or pathology. We did not observe any alterations in levels or phosphorylation of tau following treatment. These results suggest that the bioactive compounds derived from *Af* may have modest benefits to AD.

MATERIALS AND METHODS

Mice

All animal experiments were approved by the University of Illinois at Chicago Institutional Animal Care and Use Committee (IACUC). APP^{swe}/PS1^{E9} have been previously described [17]. Animals were maintained in standard conditions (14/10 h light/dark cycle) with full access to food and water *ad libitum*. Only female mice were used in this study.

Plant material and isolation of active compounds

Achillea fragrantissima (Forssk) Sch. Bip (*A. fragrantissima*) was collected in the Arava Valley by Dr. Rivka Ofir. The plant was authenticated by the botanist Mrs. Mimi Ron, The Mount Scopus Botanical Garden in The Hebrew University of Jerusalem, and the voucher

specimen has been kept as part of the Arava Rift Valley Plant Collection under the accession code AVPC0040. Achillolide A was isolated as previously described [13]. TTF was isolated as previously described [15]. Each of the purified compounds was then dissolved in DMSO at a concentration of 25 mg/mL.

Treatment

Mice were injected daily via intraperitoneal injection with either 25 µg AcA or 25 µg TTF in a 0.7% saline solution of 100 µL total volume for 28 days. An equivalent volume of DMSO was administered in 100 µL of 0.7% saline solution as the vehicle control. The treatment began at 6 months of age and lasted for 28 days.

Contextual fear conditioning

APPswe/PS1 E9 mice treated with DMSO, AcA, or TTF were placed into a 17.8 × 17.8 × 30.5 cm chamber with two clear Plexiglass walls, two metal walls, and a stainless-steel grid floor (Coulbourn Instruments). The chamber was located inside an opaque isolation cabinet. The mice remained in the cage for 148 s before receiving a single 0.75 mA foot shock lasting 2 s. The mice remained in the cage for another 30 s after the shock before being returned to their home cage. Twenty-four hours later the mice were returned to the chamber for 5 min. Freezing was measured using a digital video camera mounted above the chamber and FreezeFrame software (Actimetrics). Percentage freezing time was analyzed using FreezeView software (Actimetrics).

Novel Object Recognition

APPswe/PS1 E9 mice treated with DMSO, AcA, or TTF were habituated to the experimental arena for 5 min for 2 consecutive days. On the third day, the mice were presented with two identical objects and were allowed up to 20 min to explore for a cumulative time of 30 s (“familiarization”). On the fourth day, a new object was placed on the less-preferred side of the familiarization day and the mice were again allowed up to 20 min to explore for a cumulative time of 30 s. Exploration was defined as the nose pointed at the object in the area immediately surrounding the object. Mice that did not reach a cumulative total of 30 s of exploration time during the familiarization or Novel Object Recognition tasks were excluded from the analysis. Nose tracking and timing was performed using Noldus EthoVision software.

Protein extraction for Western blot, dot blot and ELISA analysis

Following behavior experiments, mice were sacrificed and the hippocampus and cortex flash frozen in liquid nitrogen. Protein was extracted in a buffer containing 50 mM Tris pH7.2, 150 mM NaCl, 5 mM EDTA, and 1% SDS and supplemented with protease and phosphatase inhibitors (2 mM sodium orthovanadate, 50 nM RR microcystin, 50 nM okadaic acid, 100 nM K52a, 100 nM staurosporine, 1 µL/100 µL buffer mammalian protease inhibitor cocktail II (Sigma P8340), 1 µL/100 µL buffer potassium phosphatase cocktail II (Millipore 524625), and 1 mM PMSF. Samples were homogenized on ice using a Dounce homogenizer and centrifuged for 15 min at 10,000 × g at 4°C. The supernatant was collected and protein quantified using a BCA protein assay (ThermoScientific). For western blot, samples were

separated on a 7.5% Tris-glycine gel and transferred to a 0.45 μ m nitrocellulose membrane (Biorad) at 100 mV for 2 h at 4°C. Membranes were then blocked for 1 h in 5% milk in PBST (PBS+0.1% Tween-20). Membranes were incubated overnight with primary antibodies in the 5% milk solution at 4°C with constant rotation. The membranes were then washed three times for 10 min each in PBST with shaking and then incubated with the appropriate secondary antibody for 1 h at room temperature in 5% milk with constant rotation. The membranes were then washed again 3 times for 10 min each in PBST with shaking. Finally, the membranes were imaged using an ECL chemiluminescence substrate (GE Health-care). Relative levels of protein were quantified using densitometric measurements from ImageJ software. For dot blot analysis, 75 μ g of total protein was loaded into each well of a dot blot apparatus (BioRad) and directly blotted onto a 0.45 μ m nitrocellulose membrane (Biorad). Three repetitions of each sample were loaded into the apparatus. The membrane was then treated as in the western blot. The antibodies used include: rabbit anti A β PP C-terminus (1 : 2,000, Abcam ab2072); 22C11 (Millipore MAB348 1 : 2000); anti-A β ₁₋₁₇ clone 6E10 (1 : 2500, BioLegend 803001); mouse anti Actin (1 : 5,000, Millipore MAB1501); rabbit anti-amyloid Oligomer (A11, 1,1 : 000, Millipore AB9234); mouse anti-Tau5 (1 : 1,000, Millipore MAB361); AT8 mouse anti-phospho-tau (Ser202, Thr205), (1 : 1,000, ThermoFisher MN1020); mouse anti-phospho-tau monoclonal antibodies CP13 (pSer202), RZ3 (pThr231), PHF1 (pS396, S404), generously provided by Dr. Peter Davies [18]. Levels of A β ₄₀ and A β ₄₂ were detected using Human A β solid-phase sandwich ELISA, (Invitrogen KHB3481) for A β ₄₀ and (Invitrogen KHB3441) for A β ₄₂.

Statistical analysis

For the Familiarization and Novel Object Recognition behavior analysis, a paired, two tail t test was used (DMSO N = 5, AcA N = 8, TTF N = 8). For Con-textual Fear Conditioning, a one-way ANOVA was used (DMSO N = 8, AcA N = 8, TTF N = 8). For western blot analysis, a one-way ANOVA was used with follow up unpaired two-tailed t test when applicable. For A β PP and C-terminal fragments DMSO N = 8, AcA N = 8, TTF N = 8. For oligomeric A β , DMSO N = 3, AcA N = 3. For AT8, DMSO N = 11, AcA N = 11, TTF N = 8. For Tau5 DMSO N = 11, AcA N = 11. Statistical analysis of western blot analysis using cell lines was performed by unpaired two tailed test, and non-parametric Mann-Whitney test, N =3 for TTF, ACA and their matching DMSO-treated control samples.

Cell lines

Human Embryonic Kidney (HEK) cells over-expressing FAD-linked Swedish mutant form of amyloid precursor protein (HEK-APP^{swe}) were cultured in a 10 cm dish in HEK media-DMEM (1X)+GlutaMAX (1X) media (Life Technologies 10569–010) supplemented with 10% FBS (Life Technologies 10439024), 1% Pen-Strep (Fisher Sci 15140–122), and Geneticin (Life Technologies 10131–035) (20 μ L per 10 mL). The media was changed every 3–4 days and the cells were split once a week. At 80%–90% confluency, cells were incubated with 8 μ M TTF or 100 μ M AcA treatments for 72 h. Concentrations were chosen based on previous studies [13, 15]. After treatment, the cells were incubated in HEK media for 3 hours and both the supernatant and cell extract were collected for further analysis.

RESULTS

The effect of AcA and TTF on the performance of FAD mice in learning and memory tasks

Female APPswe/PS1 E9 mice exhibit learning and memory impairments in hippocampus-dependent tasks such as contextual fear conditioning and Novel Object Recognition as early as 3 months of age [19], though widespread plaque deposition in the hippocampus is typically observed between 4–6 months of age [20, 21]. We asked if treatment with AcA or TTF could ameliorate cognitive impairments on these tasks in the presence of plaque pathology. After treating 6-month-old female APPswe/PS1 E9 mice with either AcA or TTF for one month, we began behavioral testing while continuing daily injections (Fig. 1A). The first behavior task we performed was Novel Object Recognition, in which the mice are familiarized to two identical objects, and then presented with a novel object on the following day (Fig. 1B). The percentage of time spent exploring the familiar and novel object is measured, and a greater percentage of time spent with the novel object indicates that the mouse remembers, and is therefore less interested, in the familiar object. This task depends on the hippocampus and cortex, and we have previously demonstrated that female APPswe/PS1 E9 mice exhibit impairments in this task as early as 3 months of age [19]. While the DMSO vehicle-treated mice had no preference for the novel object, mice treated with either AcA or TTF exhibited a trending preference for the novel object (Fig. 1C, D). Next, we performed a secondary behavior task, contextual fear conditioning. In this task the mice learn to associate a context with a mild foot shock. The amount of freezing is measured when the mice are reintroduced to the context, with a greater amount of freezing indicating that a memory has been formed (Fig. 1E). We did not observe any difference in performance in the contextual fear conditioning task between mice treated with either AcA or TTF and mice treated with DMSO vehicle control (Fig. 1F). These results suggest that the impact of AcA and TTF treatment on cognitive function is modest and may be task-specific. Future experiments may need to increase power to account for the small effect size, or utilize different cognitive tasks that may be more sensitive to the cognitive effects of these compounds.

AcA and TTF enhance full length A β PP without exacerbating amyloidogenic processing in vivo

Following the behavior experiments, we asked if the compounds AcA or TTF would affect the neuropathological hallmarks of AD. To start to address this we first asked whether AcA or TTF modulate A β PP in the brains of APPswe/PS1 E9 mice. These mice overexpress A β PP and have increased levels of A β [22]. Interestingly, we observed upregulation of full length A β PP (observed at approximately 110 kDa using Abcam ab2072) in the cortex of 6-month-old APPswe/PS1 E9 mice following one month of treatment with either AcA or TTF (Fig. 2A, B). However, there was no change in the levels of APP-CTFa (Fig. 2C) or APP-CTF β (Fig. 2D; CTFs were detected between 10–15 kDa using Abcam ab2072). To further examine the possibility that increased levels of full length A β PP (FL-APP) lead to more amyloidosis, we examined levels of oligomeric A β (Millipore AB9234) in the brains of these mice. We did not observe any difference in the level of oligomeric A β between AcA-treated and vehicle-treated brains (Fig. 2E, F). Taken together, these results suggest that AcA or TTF do not exacerbate the amyloidogenic processing of A β PP. This may imply that the

AcA and TTF compounds stabilize A β PP rather than enhance amyloidogenic processing. Thus, we propose that these compounds may be natural modulators of A β PP processing or of μ -secretase activity.

AcA and TTF do not affect the development of tau pathology in vivo

To examine the possibility that AcA or TTF would affect tau metabolism or pathology, we examined levels of AT8 immunoreactive p-tau in brain extracts of APP^{swe}/PS1^{E9} mice following treatment with either AcA, TTF, or vehicle, and total tau expression following treatment with AcA or vehicle, using western blot analysis. We did not observe any alterations of p-tau (ThermoFisher MN1020) expression (Fig. 3A, B) or total tau (Millipore MAB361) expression (Fig. 3C, D) following treatment. This may be attributed to the relatively young age of the mice. However, it should be noted that while reduced levels of AT8-positive p-tau were observed in the brain extracts of these mice following environmental enrichment, [23] the extent of tau pathology in this mouse model is very modest. Thus, future studies should examine the effect of these compounds on tau pathology in mouse models that develop neurofibrillary tangles, such as the 3XTg-AD mouse model [24].

The effect of TTF and AcA on the amyloidogenic pathway and tau phosphorylation in vitro

To further understand the effect of these compounds on A β PP and tau metabolism, Human Embryonic kidney cells overexpressing APP^{swe} (HEK-APP^{swe}) were treated with either TTF (8 μ M) or ACA (100 μ M), or the corresponding DMSO control for 72 h. After treatment, the cells were incubated in HEK media for 3 h and both the supernatant and cells extract were collected for further analysis. Following treatment with TTF, levels of full length A β PP were significantly increased in treated cells compared to control (Fig. 4A, B). There was no difference in the levels of APP-CTF α (Fig. 4A, C), APP-CTF β (Fig. 4A, D), or their ratio (Fig. 4A, E). In addition, there was no difference in levels of sA β PP α (Fig. 4A, F) or soluble A β (Fig. 4A, J). Quantification of the concentration of A β _{40,42} using ELISA revealed upregulation of A β ₄₀ (Fig. 4K) and a trending, albeit statistically insignificant, increase in A β ₄₂ (Fig. 4L). Thus, there was no change in the ratio of A β _{42,40} (Fig. 4M). Taken together, these results suggest that while levels of A β PP are increased, A β PP processing and metabolite production do not change.

Next, we determined the effect of TTF on tau phosphorylation. We found that the levels of CP13 were significantly higher in TTF treated cells compared to control (Fig. 4A, G). However, there was no change in reactivity level to PHF1 and RZ3 (Fig. 4A, G–I). This may suggest that TTF may affect the phosphorylation of select tau epitopes.

Further, we examined the effect of AcA treatment on A β PP metabolism in HEK-APP^{swe} cells. Similar to the previous results, we observed a significant upregulation in the levels of FL-A β PP compared to DMSO control (Fig. 5A, B) and a trending increase in sA β PP α ($p = 0.16$) (Fig. 5A, C). A significant increase was observed in the levels of CTF α , with a trending increase in the level of CTF β and thus, a trending, albeit statistically insignificant, increase in the APP-CTF α β ratio (Fig. 5A, D–F). No difference was observed in the expression levels of A β or concentration of A β _{40,42} between treated and control cells by

Western blot analysis or ELISA, respectively (Fig. 5A, I–L). No difference was found in the levels of RZ3 and CP13 (Fig. 5A, G, H). This data suggests that like TTF, AcA treatment increases expression levels of full length A β PP, but does not enhance its amyloidogenic processing. In contrast to TTF, treatment with AcA resulted in some trending increase in products of α -secretase. Taken together, our *in vitro* data supports our hypothesis that these compounds stabilize A β PP rather than enhancing its amyloidogenic processing.

DISCUSSION

This study provides evidence that AcA and TTF affect the expression level of A β PP. This observation suggests that these small molecules may modulate A β PP function. This study presents a thorough investigation of the effect of AcA and TTF on A β PP processing in FAD-linked mice and in a FAD-linked cell line and shows that AcA and TTF increase A β PP expression while they do not facilitate its amyloidogenic processing. There are several ways by which A β PP expression can be regulated. First, it is possible that AcA and TTF stabilize the anchoring of A β PP in the membrane. The second possibility is that they prolong the half-life of A β PP. Third, they may inhibit the internalization of A β PP. Fourth, they may modulate the interaction of A β PP with one or more of its cleaving secretases. Fifth, they may modulate posttranslational modification of A β PP. Notably, a previous study suggests that the phosphorylation of A β PP₆₉₅ on the S655 residue positively modulates its secretory trafficking [25]. Further studies are warranted in order to determine the mechanism by which AcA and TTF regulate A β PP levels.

Notably, increased levels of A β PP takes place during neurogenesis [26]. There, A β PP increases selectively and progressively during neuronal differentiation but is not processed in significant amounts by secretory cleavage. Thus, differentiation of neurons is accompanied by an increased A β PP level and membrane retention of the protein as the intact, full-length form [26]. A physiological increase in A β PP level has also been recently described to be modulated to different extents by Apolipoprotein E (ApoE) isoforms [27]. Thus, we cannot exclude the possibility that AcA and TTF modulate the ApoE-A β PP interaction. Further studies should address this possibility. Interestingly, both compounds had similar effects on A β PP metabolism, suggesting either shared structure or function in that regard.

Second, we show that treatment with AcA and TTF very modestly improve some behavioral aspects in the APP^{swe}/PS1^{E9} mice. Given the other therapeutic characteristics of these compounds, i.e., anti-inflammatory and anti-oxidative stress effects [13, 15], we cannot exclude the possibility that a different dose or duration of treatment would have a better behavioral outcome. Further studies should examine these possibilities.

We found no consistent effect of the compound on p-tau species. However, the APP^{swe}/PS1^{E9} mice are not necessarily the appropriate mouse model for the examination of tau metabolism and pathology. These experiments should be recapitulated in other mouse models (e.g., [24]).

It should be noted that female APP^{swe}/PS1^{E9} mice experience an accelerated disease course, developing pathology more quickly and to a greater extent than their male

counterparts [28]. By using female mice, we were able to investigate the effects of the compounds on a more severe disease state more quickly. Future studies will examine the effect of these compounds on AD pathology in males.

In summary, this study examined the therapeutic potential of AcA and TTF in FAD-linked APP^{swe}/PS1 E9 mice and in HEK-APP cells. We discovered that AcA and TTF are potential modulators of A β PP metabolism. Future studies should examine the structure-function association of these compounds for drug discovery purposes.

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Fig. 1. Phytochemicals from Af modestly enhance memory in a task-specific manner. A) Schematic of experimental design. Six-month-old female APPswe/PS1 E9 mice were given daily intraperitoneal injections of either AcA, TTF, or DMSO vehicle control. Injections were given for 3 weeks prior to behavior testing, and continued daily during behavior testing. On the fourth week the mice were trained and tested first on the NOR task, followed by contextual fear conditioning, before being sacrificed and tissue harvested. B) Schematic of NOR experimental design. C, D) Female APPswe/PS1 E9 mice treated with AcA or TTF

exhibit some preference for the novel object, while DMSO vehicle control treated mice do not. C) No statistically significant difference was observed in preference between the two identical objects during the familiarization stage. D) Mice treated with AcA ($N = 8$) or TTF ($N = 8$) exhibited a trending preference for the novel object, whereas mice treated with the DMSO ($N = 5$) vehicle control did not. E, F) Mice treated with AcA or TTF do not exhibit enhanced performance in a contextual fear conditioning task. E) Schematic of the contextual fear conditioning paradigm. F) No statistical difference was observed in the amount of freezing between mice treated with either AcA ($N = 8$) and TTF ($N = 8$) and mice treated with DMSO vehicle control ($N = 8$) (One-way ANOVA $p = 0.80$).

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Fig. 2.

Ac phytochemicals enhance level of full length A β PP, but not of A β PP Carboxyl-terminal fragments. A) Representative western blot (of 2 blots, DMSO = 8, AcA = 8, TTF = 8) showing that treatment of APP^{swe}/PS1 E9 mice with AcA or TTF enhances expression of full length A β PP but not the level of A β PP C'-terminal fragments (Rabbit anti Amyloid Precursor Protein C-terminus, 1 : 2,000 Abcam ab2072). B) ImageJ quantification showing that full length APP (observed at approximately 110 kDa using Abcam ab2072) A β PP is significantly increased in the brain of female APP^{swe}/PS1 E9 mice treated with AcA ($p=$

0.01) or TTF ($p = 0.02$) compared to DMSO treated mice. No difference in expression in CTF α (C) or CTF β (D) was observed between mice treated with either AcA or TTF and DMSO. CTFs were detected between 10–15 kDa using Abcam ab2072. E, F) Treatment with AcA does not alter expression of oligomeric A β (Millipore AB9234) in the brains of 6-month old female APP^{swe}/PS1 E9 mice. E) Dot blot image (run in triplicate). F) ImageJ quantification of oligomeric A β dot blot (DMSO $N = 3$, AcA $N = 3$).

Fig. 3.

Af phytochemicals do not change expression or phosphorylation of tau. A, B) Treatment with AcA or TTF does not alter expression of phosphorylated tau in the brains of female APP^{swe}/PS1^{E9} mice. A) Representative blot (of 3 blots; DMSO $N=11$, AcA, $N=11$, TTF $N=8$). B) ImageJ quantification showing that AT8 (ThermoFisher MN1020) is not changed following treatment with AcA or TTF (one-way ANOVA $p=0.22$). C, D) Treatment with AcA does not alter expression of total tau (Millipore MAB361). C) Representative blot (of 3 blots; DMSO $N=11$, AcA, $N=11$). D) ImageJ quantification showing that Tau5 is not changed following treatment with AcA (two-tailed, unpaired t-test $p=0.96$).

Fig. 4.

The effect of TTF treatment on AβPP processing and tau metabolism in-vitro. A) Representative western blot ($N=3$) showing the effect of 8 μ M TTF treatment on AβPP and tau metabolites in Human Embryonic Kidney cells overexpressing the FAD-linked Swedish mutant form of amyloid precursor protein (HEK-APPswe). B-J) ImageJ quantification of expression levels shown in (A). Full length AβPP (FL-APP, Abcam ab2072) is significantly increased ($p=0.0023$) (B). No significant difference was observed in the levels of AβPP-CTFα (C) or APP-CTFβ (D) or in the APP-CTFα/β ratio (using Abcam ab2072) (E).

Likewise, no difference in the levels of soluble A β PP α was observed (F). A significant increase was observed in the levels of tau metabolite CP13 ($p = 0.032$) (G) with no difference in the levels of PHF1 and RZ3 metabolites (H,I). No change in the expression of soluble A β was observed ($p = 0.16$, Biolegend 803001) (J). K-M) Quantifications of the levels of A β fragments (A β_{40} and A β_{42}) using Thermo Fisher Human A β ELISA kits (KHB3481, KHB3441) shows a significant increase in A β_{40} (K), but no significant change in A β_{42} (trending increase, $p = 0.18$) (L) or the ratio A $\beta_{42/40}$ (M).

Fig. 5.

The effect of AcA treatment on AβPP processing and tau metabolism in-vitro. A) Representative western blot ($N=3$) showing the effect of 100 μ M AcA treatment on AβPP and tau metabolites in HEK-APPswe cells. B-I) ImageJ quantification showing that full length AβPP (FL-APP Abcam ab2072) is significantly increased ($p = 0.005$) following treatment (B). A trending increase, albeit insignificant ($p = 0.16$), in the levels of AβPPα was observed following treatment (C). A significant increase in the levels of APP-CTFα was observed ($p = 0.01$) (D). No change in the levels of APP-CTFβ (E) or APP-CTFαβ ratio

was observed (F). G, H) No difference in tau phosphorylation was observed as detected by CP13 and RZ3 antibodies. I) No change in the levels of soluble A β (I) or concentration of A β_{40} (J) and A β_{42} (K) or their ratio (L) were observed, as determined by Thermo Fisher Human A β ELISA kits (KHB3481, KHB3441).

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