The Effect of Curcumin on Aβ, Akt, and GSK3β in Brain and Retina of APP/PS1 Mice and Blood of Alzheimer's Patients With Early Stage Disease

Xi Mei (meixi18401856@163.com)
Ningbo Kangning Hospital
Conglong Qiu
Ningbo Kangning Hospital
Lingli Shi
Ningbo Kangning Hospital
Xingxing Li
Ningbo Kangning Hospital
Mengxiang Yang
Ningbo University
Jun Hu
Ningbo Kangning Hospital
Zhongming Chen
Ningbo Kangning Hospital
Chenjun Zou
Ningbo Kangning Hospital

Research

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Abstract

Background

Curcumin has multifunctional pharmacological properties, including anti-oxidant, anti-inflammatory, and anti-diabetic properties. We investigated whether curcumin can improve pathological changes associated with Alzheimer's disease, including amyloid β (Aβ), protein kinase B (PKB, also termed Akt), and glycogen synthase kinase 3β (GSK3β) levels and expression.

Methods

Alzheimer's transgenic $APP_{SWE}/PS1_{\Delta E9}$ mice and wild type mice were treated with curcumin by intragastric administration for 2 weeks at 2 and 5 months of age. Aβ plaques and contents in the brain and retina were measured by immunohistochemistry and enzyme-linked immune sorbent assay, respectively, while the expression of Akt and GSK3β was tested by RNA isolation and quantitative real-time poly merase chain reaction. Bloods of patients with AD and age-matched health controls were used to determine the contents of Aβ, Akt and GSK3β.

Results

Curcumin treatment decreased Aβ accumulation in the early stages of AD at 5 months ($p < 0.001$). It also improved AD-associated pathological changes, including upregulation of Akt ($p < 0.01$) and downregulation of GSK3β ($p < 0.01$). In addition to AD-associated changes, the proinflammatory cytokine IL-1β was significantly decreased by curcumin treatment ($p < 0.05$).

Conclusions

Curcumin can suppress Aβ accumulation during the early stages of AD, upregulate the expression of Akt, downregulate the expression of GSK3β, and inhibit the proinflammatory cytokine IL-1β. Curcumin can be used to improve pathological features of AD in the early stages of disease

Introduction

In Alzheimer's disease (AD), accumulation of amyloid β (Aβ) is one of the key processes in disease progression and a key mediator of synaptic dysfunction and cognitive impairment [1]. Aβ regulates protein kinase B (PKB, also termed Akt) and glycogen synthase kinase 3β (GSK3β) signaling, which interact with each other in the AD brain [2,3]. Additionally, Aβ production may also be regulated by GSK3 [4].

Previous studies have suggested that the AKT/GSK-3b pathway plays a key role in the maintenance of neuronal survival and neuronal networks in AD [5,6]. Pharmacological activation of Akt rescued memory impairment in Aβ-injected AD model mice [7]. Disturbance of the AKT/GSK-3b signaling pathway is a key

mechanism underlying the pathophysiology of both neurodegenerative diseases and diabetes mellitus [8,9].

Curcumin is the main active component of the traditional Chinese medicine turmeric, which has anti-inflammatory, anti-oxidant, and anti-tumor effects [10]. Previous curcumin human tolerance tests and clinical research on the effect of curcumin treatment of AD have shown that the probability of digestive tract intolerance is very small [11].

Animal experiments have shown that after one week of curcumin treatment, the number of Aβ plaques in the brains of $APP_{SWE}/PS1_{\Delta E9}$ (APP/PS1) transgenic mice was significantly reduced [12,13]. The ability of curcumin to effectively clear Aβ is likely due to ability to breakdown products within the body.

In recent years, the neuroprotective effect of curcumin has become a research focus. It has been found that curcumin can improve cognition and inhibit inflammation by regulating related cell pathways, including regulating BDNF and Akt/GSK3β signaling pathways to improve the cognitive decline of rats caused by intracerebroventricular injection of $Aβ_{42}$ [14].

Studies into curcumin treatment of spinal injuries shows that curcumin can regulate the TLR4/NF-κB signaling pathway by downregulating the levels of TLR4, NF-κB, and inflammatory cytokines, thereby protecting against spinal injury [15]. In Aβ-stimulated microglia, curcumin can block NF-κB, JNK, and p38 MAPK signaling pathways, which produce anti-inflammatory effects, and protect hippocampal HT-22 cells from the neurotoxic effects caused by microglial activation [16]. Curcumin can also regulate the PTEN/Akt/GSK3β pathway and inhibit tau hyperphosphorylation caused by Aβ in SH-SY5Y cells [17]. Curcumin can inhibit inflammatory gene expression and pro-inflammatory pathways by inhibiting NF-κB activity [18].

To date, many studies have reported that curcumin improves cognitive function by inhibiting Aβ accumulation in APP/PS1 mice [19]. However, it remains unclear whether curcumin can prevent neuronal injury via the AKT/GSK-3b signaling pathway. In the present study, we investigated the effect of curcumin on Aβ in vivo and the protein expression of AKT and GSK-3b by immunohistochemistry and real-time quantitative polymerase chain reaction (RT-qPCR) analysis.

Materials And Methods

Animals

APP/PS1 transgenic mice and age-matched wild-type (WT) mice were provided by the Model Animal Research Center of Nanjing University (Nanjing, China). To exclude the effect of sex on the results, only male mice were used. Animals were housed in cages in a room maintained at 22±2°C and 60±5% relative humidity under a 12-h light–dark cycle (lights on at 6:00 am). Water and food were available ad libitum. Animal experiments were conducted outside the housing area in a separate room.

Patients

Nineteen AD patients and twenty age-matched healthy controls (HCs) were recruited for the study. AD patients with no retinal diseases aged between 60 and 88 years were diagnosed by two research psychiatrists according to the standards of the National Institution of Neurologic and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association, NINCDSADRDA and Diagnostic and Statistical Manual of Mental Disorders, DSM.

Drug preparation and administration

Curcumin (pure curcumin ≥ 80%, Hushi, Shanghai, China) was dissolved in phosphate buffered saline (PBS, 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4, 0.1 M KCl, and 0.1 M NaCl, pH 7.4). Animals were divided at the age of 2 and 5 months into four experimental groups (n = 15 mice/group): (1) APP/PS1 mice treated with curcumin (0.1 mg/g) dissolved in PBS; (2) APP/PS1 mice treated with a similar volume of only PBS; (3) WT mice treated with curcumin (0.1 mg/g) dissolved in PBS; and (4) WT mice treated with a similar volume of only PBS. All treatments were administered intragastrically for two weeks.

Aβ immunohistochemistry

Briefly, after anesthetization, mice were perfused with saline until the limbs and the liver turned white, and then perfused with 4% paraformaldehyde until the tail became stiff. The brain tissue was dissected and incubated with 4% paraformaldehyde for 1 d. After washing with PBS, the tissue was placed in a centrifuge tube containing 30% sucrose solution until the brain tissue sunk to the bottom. A cryostat was used to cut the brain tissue into 25 μm thick sections. The sections were incubated in 1% bovine serum albumin (BSA) for 1 h and then incubated with β-amyloid antibody (1: 500, Cell Signaling Technology) at 4°C overnight. The sections were washed three times with PBS, rinsed, and incubated with the secondary antibody at 37°C for 1 h. After staining with 4,6-diamidino-2-phenylindole (DAPI) for 1 min, the sections were washed three times with PBS and imaged using a confocal fluorescence microscope

Enzyme-linked immune sorbent assay (ELISA)

The Aβ and IL-1β levels in the mouse brain, retina, and human blood were measured by ELISA (MyBioSource, CA, USA). Absorbance at 450 nm (at a reference wavelength of 690 nm) was measured using an absorbance reader (Sunrise™, Tecan, Geneva, Switzerland). The absorbance value was transformed into a concentration value by reading the absorbance of pure samples on a standard curve.

RNA isolation and quantitative real-time polymerase chain reaction (RT-qPCR)

Akt and GSK3β expression was determined by RT-qPCR. Total cellular RNA was isolated using RNAiso Plus reagent (Takara Bio Inc., Otsu, Japan) according to the manufacturer's instructions.

Determination of serum Aβ, Akt, GSK3β, and IL-1β levels

Serum Aβ, Akt, GSK3β, and IL-1β levels were estimated using an ELISA assay kit (MyBioSource, CA, USA). All procedures were performed according to the manufacturer's instructions.

Mini-mental state examination (MMSE)

MMSE, which involves scores ranging from 0 to 30, was adopted to evaluate the cognitive levels of all subjects. Lower MMSE scores indicate poorer cognitive performance.

Statistical analyses

Data are presented as the mean ± standard error. Prism v7.0 (GraphPad, San Diego, CA, USA) was used for statistical analyses. Differences among multiple mean ± SE values were assessed by one-way and two-way ANOVA, followed by Bonferroni's *post hoc* test. Differences between two mean ± SE values were assessed using unpaired *t-tests*. Statistical significance was set at $P < 0.05$.

Table1 Demographic and clinical characteristics of all subjects

Variables	AD patients (n=9)	Health controls (n=10)	p value
Age (year)	72.56±10.27	73.91±7.82	0.368
Sex (male)	5	6	-
BMI	21.89±2.78	21.64±2067	0.815
Education years	10.33±3.08	10.46±2.58	0.505
MMSE	10.89±7.52	27.82±1.78	0.001

Results

Curcumin reduced Aβ protein levels in AD mice

Aβ plaques were stained by immunohistochemistry (Figure 1), and the levels of total Aβ in the cortex analyzed using a specific sandwich ELISA are shown in Figure 2. As shown in Figure 1, curcumin treatment reduced the amount of positive staining for Aβ in the cortex of APP/PS1 mice at 5 months, which represents the early stage of AD.

There was no obvious effect of curcumin on Aβ levels in the 2 month old mice (Figure 2). However, in the 5 months old mice, curcumin treatment decreased the Aβ levels in both the cortex and retina of the APP/PS1 group ($p < 0.05$).

Curcumin modulate the Akt and GSK3β expressions in AD mice

Compared to WT, the gene expression of Akt was significantly decreased ($p < 0.01$), whereas the gene expression of GSK3β was significantly elevated ($p < 0.05$) in the brain and retina of APP/PS1 mice

(Figure 3). After administration of curcumin, the APP/PS1 mice showed a significant decline in the expression of GSK3β at 2 months ($p < 0.01$) and elevation of Akt gene expression at both 2 and 5 months ($p < 0.01$).

Effect of curcumin on inflammatory mediators in AD mice

Levels of IL-1β measured from the soluble fraction of the brain and retina of mice at 2 and 5 months are shown in Figure 4. Compared to the WT group, APP/PS1 mice showed significantly elevated levels of IL-1β ($p < 0.001$). Moreover, compared to the APP/PS1 group, the curcumin treatment group exhibited a significant decrease in IL-1β levels ($p < 0.001$).

Difference in Aβ, Akt, GSK3β, and proinflammatory cytokines between AD patients and health subjects

The demographics and clinical characteristics of the study subjects are detailed in Table 1. Mean age of AD was 72.56 ± 10.27 years, 55.56% were male, BMI was 21.89 ± 2.78, education was 10.33 ± 3.08 years, MMSE score was 10.89 ± 7.52 and mean disease course was 7.22 ± 2.82 years. There were significant differences in the mean MMSE scores between the AD and HC groups ($p < 0.01$). There were no differences in age, BMI, or education level between the groups ($p > 0.05$).

Figure 5 shows the levels of Aβ, Akt, GSK3β, and IL-1β in the serum of AD patients and healthy controls. There was a significant difference between groups in Akt levels ($p < 0.05$), however no difference was found in Aβ, GSK3β, and IL-1β.

Discussion

Neurodegenerative diseases can affect both the brain and the retina. Although the neuroprotective effect of curcumin has already been studied, it was not yet known whether curcumin can directly influence Aβ accumulation, proinflammatory cytokines, and related proteases. The current study investigated the effect of curcumin on Aβ, Akt, and GSK3β in the brain and retina of APP/PS1 mice.

The ageing process can cause a large amount of Aβ accumulation may disrupt the immune response and produce local inflammation[8]. Additionally, Aβ clearance can be decreased due to insulin resistance [20]. Using APPswe/PS1dE9 transgenic mice, we found that Aβ plaques were observed largely at 5 months compared to 2 months. We noticed that WT mice also had high amount of Aβ in the cortex with mouse age. Aβ content increased, but Aβ plaques have not yet formed. Similarly, the pro-inflammatory cytokine IL-1β also increased with mouse age. Previous studies have suggested that sustained inflammation results in chronic Aβ deposition, which eventually leads to AD [21]. In patients with insulin resistance, there may be an inflammatory microenvironment.

GSK3β plays a key role in insulin resistance. One of the features of insulin resistance is the impaired insulin/P13K/Akt pathway, which leads to an increase in GSK3β [22]. In APP/PS1 mice, the gene expression of GSK3β was significantly increased ($p < 0.05$) in the brain and retina, whereas gene expression of Akt was significantly decreased ($p < 0.01$). Besides its role in AD, inhibition of GSK3β

activity can also prevent pathological changes in diabetes mellitus [23]. There is evidence to suggest that AD is the third type of diabetes mellitus. GSK3β was highly expressed in the human data from this study, which is in line with previous works [24,25]. The therapeutic potential of GSK3β inhibition has been investigated in several preclinical AD studies, which show that levels of tau phosphorylation and Aβ deposition can be suppressed by GSK3β inhibitors [26,27]. Compared with normal individuals, Akt expression was significantly decreased in AD patients. Akt activator influences AD-like memory impairment and LTP impairment [7].

Curcumin has multifunctional pharmacological effects, including anti-oxidant, anti-inflammatory, and anti-diabetic properties [28-31]. The current study found an inhibitory effect of curcumin on brain and retina Aβ levels and investigated the neuroprotective mechanism and the improvement of brain diseases that may result from this effect. Curcumin can also be used as an imaging agent for Aβ plaques [13]. Therefore, in addition to the neuroprotective effects of curcumin, further research on the diagnosis and treatment of curcumin with eye brain-related diseases is required in the future [32,33].

The limitation of this study was that only changes in Aβ, Akt, and GSK3β were investigated, and not the pathway changes of Akt/GSK3β. It is important to investigate a full range of pathological pathways in the AD brain, to enable to development of more therapeutic targets for AD treatment.

Conclusion

In conclusion, this study found that curcumin appears to suppress Aβ accumulation during the early stage of AD, upregulate the expression of Akt, downregulate the expression of GSK3β, and inhibit the proinflammatory cytokine IL-1β. This suggests that curcumin treatment may be useful to improve pathological features of AD in the early stages of disease.

Abbreviations

Alzheimer's disease (AD)

amyloid β (Aβ)

protein kinase B (PKB, also termed Akt),

glycogen synthase kinase 3β (GSK3β)

APPSWE/PS1ΔE9 (APP/PS1)

real-time quantitative polymerase chain reaction (RT-qPCR)

wild-type (WT)

healthy controls (HCs)

phosphate buffered saline (PBS)

4,6-diamidino-2-phenylindole (DAPI)

Enzyme-linked immune sorbent assay (ELISA)

Mini-mental state examination (MMSE)

Declarations

Acknowledgement

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Statement of Ethics

All animal experiments were performed according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996) and were approved by the Institutional Animal Care and Use Committee of the Medical School of Ningbo University (Ningbo, China).

This study was approved by the local Ethics Committee of Ningbo Kangning Hospital (NBKNYY-2018-LC-21) and registered as a clinical trial in the China Clinical Trial Registry (CHICTR: ChiCTR2000035243). All procedures of the study were in accordance with the Helsinki Declaration (2014) ethical standards and regulations for human research.

Conflict of Interest

No competing interests

Funding

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Author Contributions

XM, XL and MY performed animal experiments, data collection and wrote the manuscript. XM, CQ, and JH performed data analysis. CZ, and ZC proofread the manuscript. All authors read and approved the final manuscript.

Data Availability Statement.

All data are included in the manuscript. However, the raw data used and/or analyzed in the present study are available from the corresponding author on reasonable request.

Consent to publish

Not applicable

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Figures

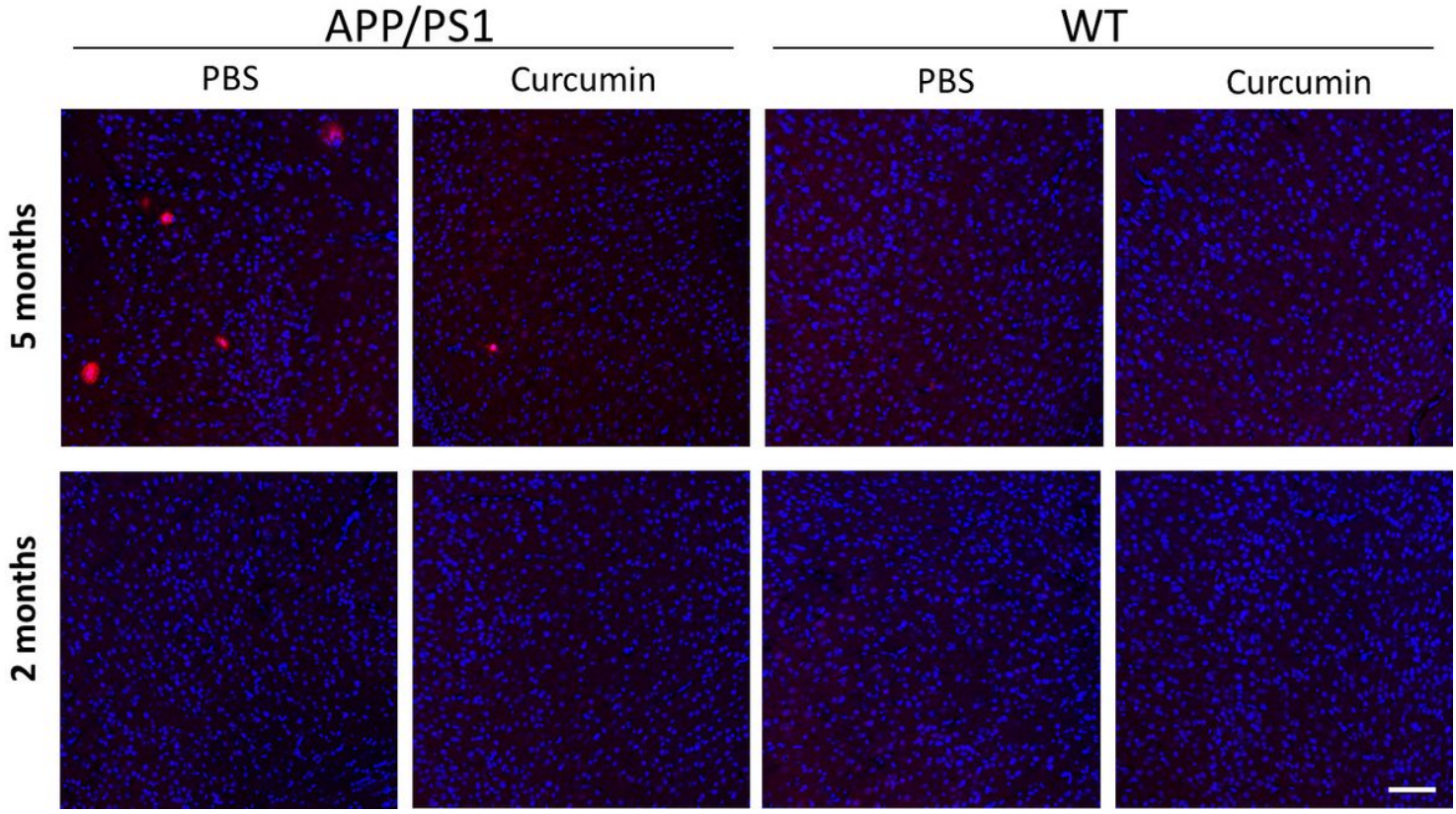

Figure 1

Immunohistochemistry in the cortex of APP/PS1 and WT mice at 2 and 5 months (Red: Aβ; Blue: DAPI). WT exhibits no positive staining for Aβ in either age group; APP/PS1 at 5 month show positive Aβ staining. APP/PS1: APPswe/PS1dE9; WT: wild type. Scale bar = 100 μm.

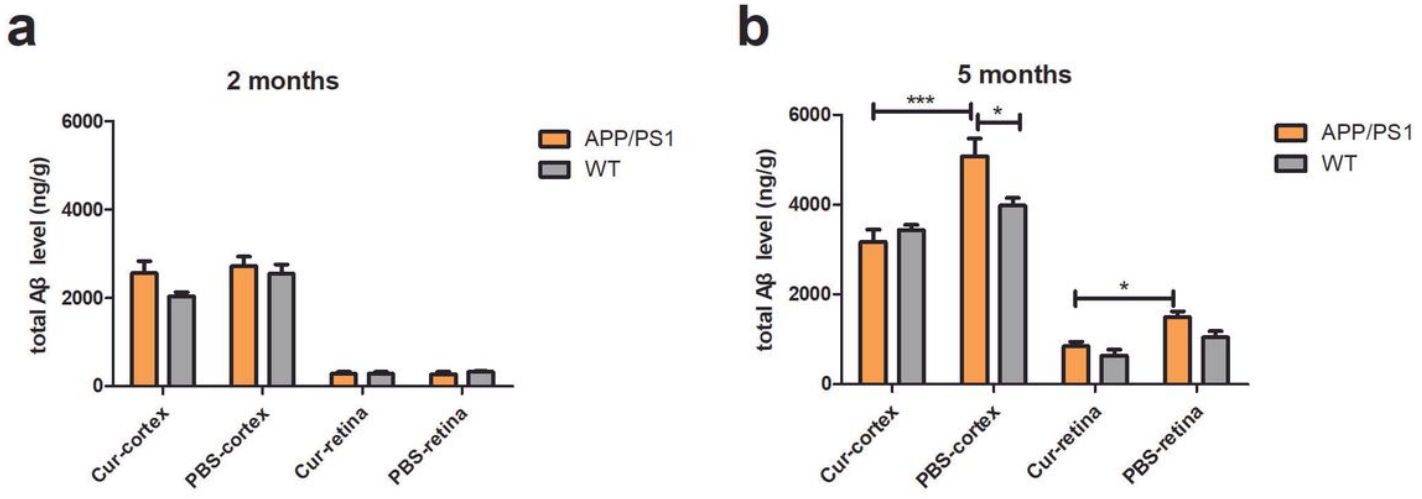

Figure 2

Aβ levels in cortex and retina of APP/PS1 and WT mice at 2 (a) and 5 (b) months. APP/PS1: APPswe/PS1dE9; WT: wild type. ***p<0.001; **p<0.01; *p< 0.05

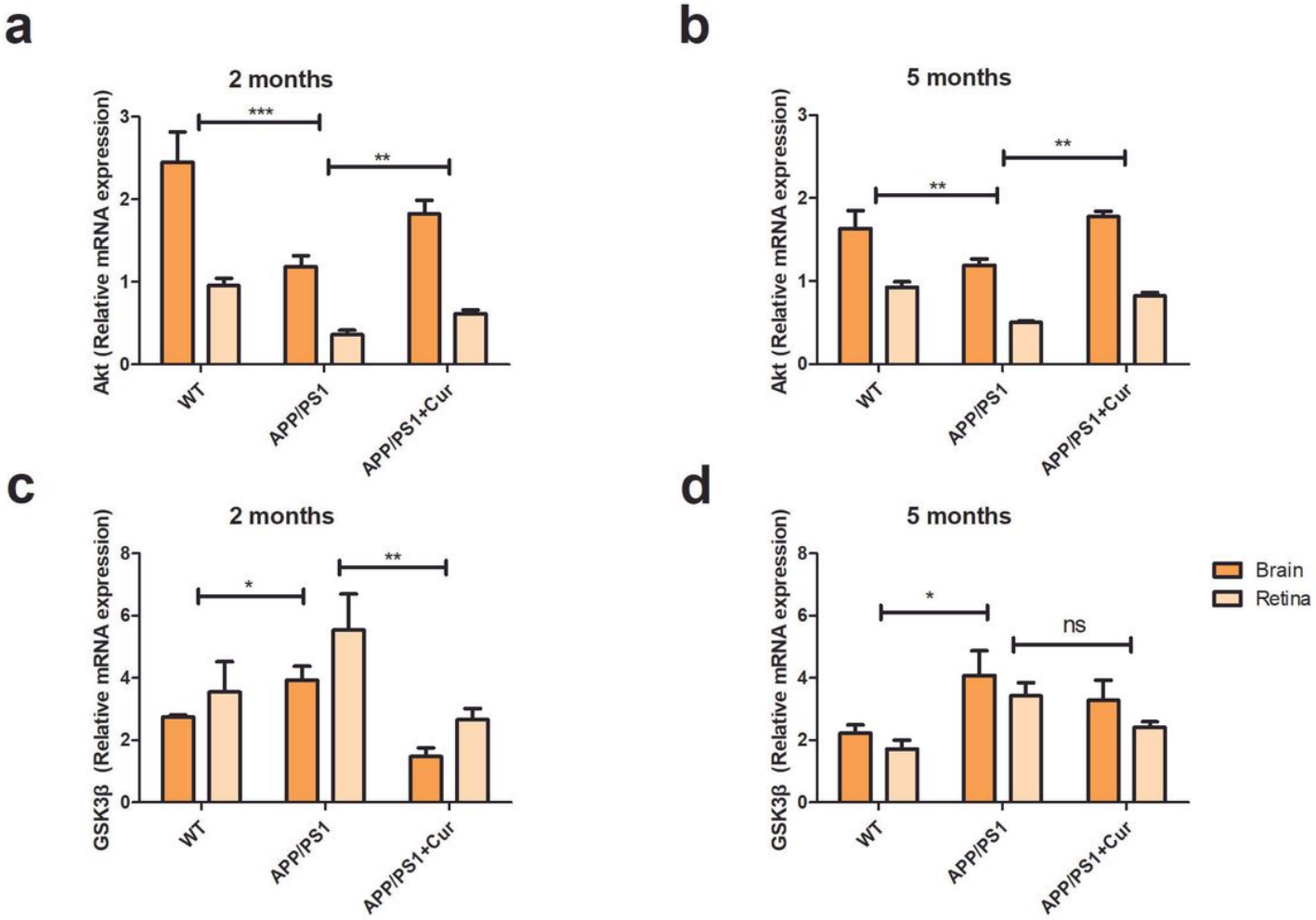

Figure 3

Effect of curcumin administration for 2 weeks on the gene expression of Akt at 2 months (a)and 5 months (b) and GSK3β at 2 months(c) and 5 months (d) in APP/PS1 and WT mice. Values represent the mean and standard deviation, using two-way ANOVA followed by Bonferroni's posthoc test. ***p<0.001; **p<0.01; *p< 0.05

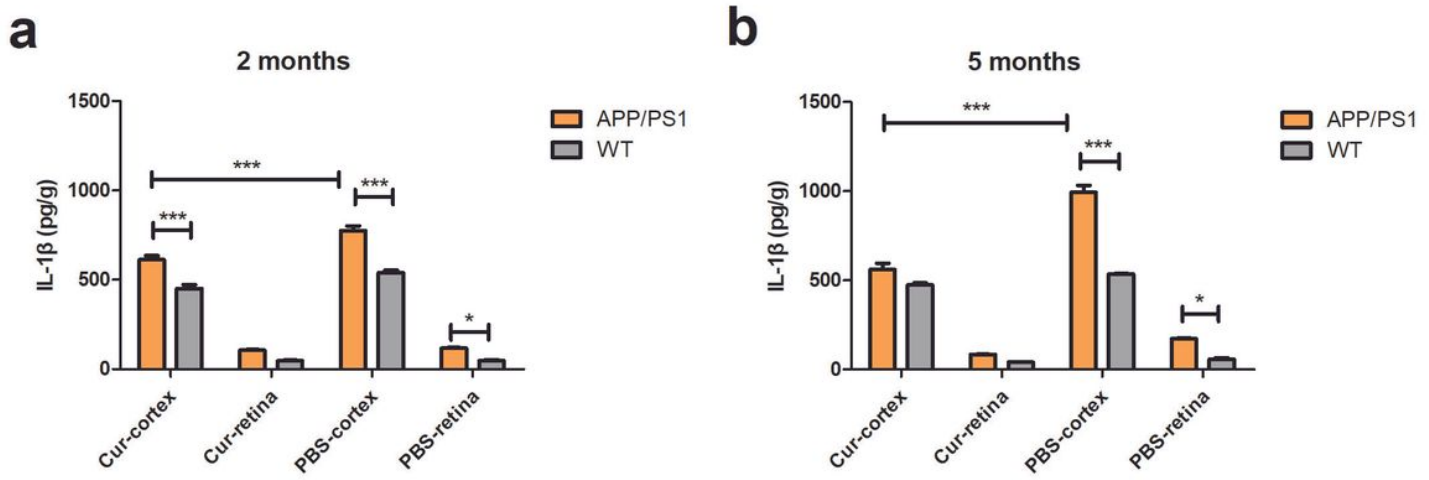

Figure 4

Curcumin shows a significant decrease in IL-1β levels in APP/PS1 mice at both 2 months (a) and 5 months (b). A two-way ANOVA (genotype × treatment) reveals a significant effect of curcumin treatment. ***p<0.001; **p<0.01; *p< 0.05

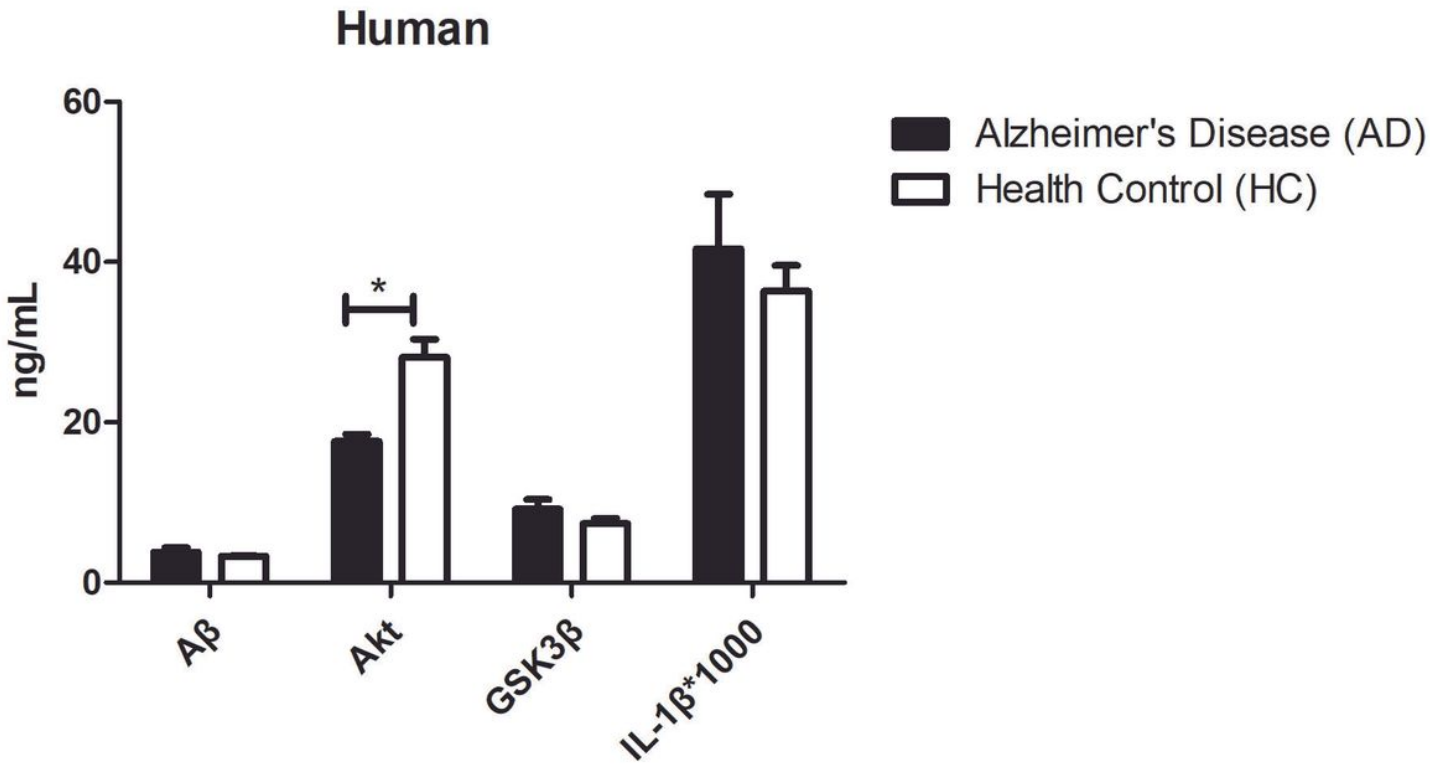

Figure 5

Levels of Aβ, Akt, GSK3β and IL-1β in serum of AD patients and health controls.