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Original article

Protective effects of curcumin on acrolein-induced neurotoxicity in HT22 mouse hippocampal cells

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ABSTRACT

Background: Aging is one of the most important inevitable risk factors of Alzheimer disease (AD). Oxidative stress plays a critical role in the process of aging. Curcumin has been proposed to improve neural damage, especially neurodegenerative injury, through its antioxidant and anti-inflammatory properties. Therefore, we investigated the effects of curcumin on acrolein-induced AD-like pathologies in HT22 cells.

Methods: HT22 murine hippocampal neuronal cells were treated with 25 μM acrolein for 24 h with or without pre-treating with curcumin at the selected optimum concentration (5 μg/mL) for 30 min. Cell viability and apoptosis were measured by CCK8 assay and flow cytometric analysis. Levels of glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) were detected by a GSH assay kit or commercial assay kits, respectively. Alterations in the expression of BDNF/TrkB and key enzymes involved in amyloid precursor protein (APP) metabolism were assessed by western blotting.

Results: Data showed that curcumin significantly reversed acrolein-induced oxidative stress indicated by depletion of GSH and SOD, and elevation of MDA. The findings also suggested curcumin's potential in protecting HT22 cells against acrolein through regulating the BDNF/TrkB signaling. In addition, acrolein-induced reduction in A-disintegrin and metalloprotease, and the increase of amyloid precursor protein, β-secretase, and receptor for advanced glycation end products were reversed either, and most of them were nearly restored to the control levels by curcumin.

Conclusion: These findings demonstrate the protective effects of curcumin on acrolein-induced neurotoxicity *in vitro*, which further suggests its potential role in the treatment of AD.

Introduction

Alzheimer disease (AD), first diagnosed by Dr. Alois Alzheimer in 1906, is characterized as an insidious, chronic, and progressively neurodegenerative and cureless disorder in the central nervous system, associated with advanced cognition deficit especially in learning and memory and accompanied by abnormal behavior and personality changes [1,2]. Although senescence, an irreversible factor, plays a critical role in the onset and progression of AD, other relatively controllable factors such as diet and environmental agents have attracted much attention, as their potent detriments in AD have been found and proved gradually [3].

Acrolein, an α,β-unsaturated aldehyde, is known as a toxin acquired from both exogenous and endogenous sources. Apart from daily exposure to environmental pollution including combustion of polyethylene plastics, industrial waste incinerators, cigarette smoking, and overheated cooking of food and oils, internal lipid peroxidation of polyunsaturated fatty acids, DNA, and proteins as well as metabolism of allyl compounds also contribute to the amount of acrolein we are exposed to [4]. Significantly increased acrolein levels in the brain and spinal cord of patients with Parkinson's disease (PD), AD, and spinal cord injury have been observed in a large body of clinical research [5,6]. Previous studies have found acrolein-induced neurotoxicity in several laboratory

Abbreviations: AD, Alzheimer disease; A, amyloid-beta peptide; ADAM-10, A-disintegrin and metalloprotease 10; APP, amyloid precursor protein; GFAP, glial fibrillary acidic protein; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; MWM, Morris water maze; NEP, neprilysin; LR-11/SorLA, sortilin-related receptor; LRP-1, low density lipoprotein receptor related protein-1; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; SOD, superoxide dismutase.

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cell lines including HT22 hippocampal cells, primary cortical neurons, and dorsal root ganglionic neurons [7,8], indicating the capacity of acrolein to induce neural damage in neurodegenerative disorders [9].

Curcumin is a yellow pigment obtained from the root of the *Curcuma longa*, a common herb used in traditional Chinese medicine. As a highly lipophilic substance, curcumin can easily cross the blood-brain barrier and then inhibit the amyloid-β peptide aggregation through binding to plaques in the brain, thus benefitting patients with AD [10]. Its strong antioxidant properties, including up-regulating the concentrations of antioxidant enzymes, reducing reactive oxygen species (ROS) generation, suppressing lipid peroxidation, and decreasing malondialdehyde (MDA) level have been reported [11–14]. In addition, curcumin was found capable of suppressing the activity of β-secretase, and then subsequently inhibiting the formation of fibril and oligomer, which finally leads to decreased plaque pathogenesis [14,15].

In terms of the aforementioned evidences, we sought to investigate the effects of curcumin on acrolein-induced neurotoxicity *in vitro*. Well-known mouse hippocampal neurons, HT22 cells, were used.

Materials and methods

Materials

Acrolein was purchased from Gelei Xiya Chemical Co. (Chengdu, China). Dullbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibico-BRL (Grand Island, NY, USA). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories (Kumamoto, Japan). Curcumin, and all other chemicals, were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. The primary and secondary antibodies used in our experiments are summarized in Table 1.

Experimental procedures

Cell culture and cell treatment

HT22 murine hippocampal neuronal cells were maintained in DMEM supplemented with 10% (v/v) FBS and incubated at 37 °C under 5% CO2. To study the cytotoxicity of acrolein, cells were planted in 96-well plates at a density of 4 000 cells per well. The fresh solution of acrolein (1 M in distilled water) was diluted in DMEM supplemented with 0.5% (v/v) FBS immediately before addition to each well at the desired final concentrations. To further investigate the potential effects of curcumin on acrolein-induced neurotoxicity, cells were pretreated with different concentrations of curcumin (0–100 μg/mL) per previous literature [16] for indicated time (6–48 h), after which cell viability was assessed by CCK-8 assay, to select the most proper concentration for the following experiments. Data showed that cells cultured in 5 μg/mL curcumin exhibited the highest cell viability; thus, 5 μg/mL curcumin was used for the following experiments. Cells in the control group were treated with vehicle alone. Referring to the concentration of acrolein corresponding to that in AD patients' brains (hippocampus 5.0 ± 1.6 nmol/mg protein and amygdale 2.5 ± 0.9 nmol/mg protein), 25 μM acrolein was used in our experiments[17]. Thus, for the experimental group, after pre-incubation with 5 μg/mL curcumin for 30 min, cells were then incubated with 25 μM acrolein for another 24 h.

Analysis for cell viability

Cell viability was measured by CCK8 according to the manufacturer's instruction. Briefly, cells were seeded into a 96-well plate at a density of 4000 cells/well in growth medium and cultured to approximately 60% to 70% confluency, prior to the initiation of experimental treatment. After receiving the designated interventions, the original medium was removed, and 10 μl of CCK-8 solution (5 mg/mL stock) was added to the cells and they were then incubated for 2 h at 37 °C. Then, absorbance at 450 nm was read with an enzyme-linked immunosorbent assay (ELISA) reader (Bio-Tek). Finally, the mean value and standard deviation of optical density for all six wells was calculated to draw the cell viability curve.

Flow cytometric analysis of apoptosis

After treatment with acrolein, cells were harvested by trypsinization and washed once with phosphate-buffered saline (PBS, pH 7.4). After centrifugation, cells were stained with annexin-V and propidium iodide (PI) using the annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit (BD Biosciences, San Jose, CA, USA) as a measurement of early apoptotic (lower right quadrant, annexin V+/PI− cells), late apoptotic (upper right, annexin V+/PI+ cells), and necrotic (upper left, annexin V−/PI+ cells) cell populations; the lower left quadrant (annexin V−/PI− cells) depicts live cells. For annexin-V and PI double staining, the procedure was performed according to the instructions of the manufacturers.

Estimation of intracellular glutathione (GSH)

GSH assay kit (Jiancheng Biochemical, Nanjing, China) was used to measure intracellular GSH concentration. Cells were collected and sonicated in 50 μl of ice-cold lysis buffer. After centrifuging at 10,000 × *g* for 15 min at 4 °C, the supernatant was collected. 50 μl of TEAM reagent was added to 1 ml of supernatant. 50 μl of sample or the standard solution provided in the kit was applied to each sample well. Then, 150 μl of the freshly prepared assay mixture was added to each of the wells containing standards and samples.

Table 1
Summary of antibodies and working conditions used in the experiments.

Antibodies	Species	Source	Dilution
Primary Antibodies			
Anti-β-actin	Rabbit, monoclonal	Cell Signaling Technology Beverly, MA, USA	1:1000
Anti-BDNF	Rabbit, polyclonal	Millipore MA,USA	1:500
Anti-TrkB	Rabbit, polyclonal	Abcam MA,USA	1:400
Anti-APP	Rabbit, monoclonal	Gene Tex USC,USA	1:2000
Anti-BACE1	Rabbit, polyclonal	Millipore MA,USA	1:500
Anti-ADAM10	Rabbit, polyclonal	Millipore MA,USA	1:1000
Anti-RAGE	Rabbit, polyclonal	Abcam MA,USA	1:1000
Secondary Antibodies			
Anti-Rabbit IgG (HP)	Goat	Cell Signaling Technology Beverly, MA, USA	1:2000
Anti-Mouse (HP)	Rabbit	Promega, Madison, WI, USA	1:10000
Anti-Goat (HP)	Donkey	Santa Cruz Biotechnology Inc.,CA,USA	1:1000

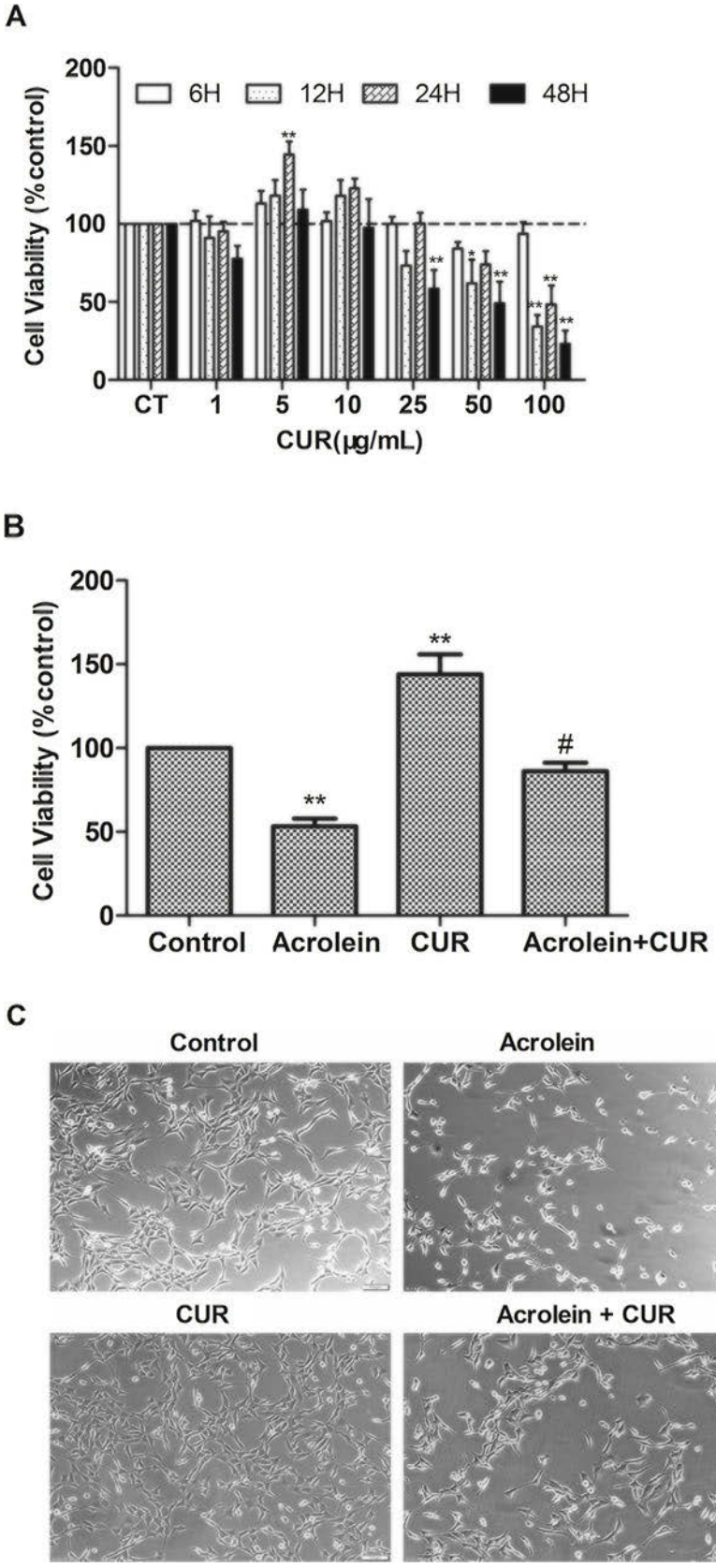

Fig. 1. Effects of curcumin on acrolein-induced neurotoxicity in HT22 cells. Cells cultured in DMEM + 0.5%FBS (v/v) medium with 5 μg/mL curcumin exhibited the highest cell viability of all groups. Curcumin (CUR) significantly reversed acrolein-induced cell death and cellular morphology destruction. Cell viability was measured by CCK-8 assay. Cellular morphology was observed by phase contrast microphotographs. Data are expressed as mean ± SEM. (A) Effects of different curcumin concentrations on HT22 cell viability at indicated time points (6–48 h). (B) Effects of curcumin on acrolein-induced neurotoxicity. $^{**}p < 0.01$ *vs.* control group; $\#p < 0.05$ (C) Phase contrast microphotographs (200×) of HT22 cells exposed to 25 μM acrolein for 24 h with or without pretreated with 5 μg/mL curcumin for 30 min.

The plate was incubated in the dark on an orbital shaker at room temperature. Absorbance was measured at 405 nm using a plate reader (Bio-Tek) after a 25-min incubation. All GSH values were normalized to per mg protein of each sample.

Assay of SOD activity and MDA level

MDA level was measured as described previously[18], using commercial assay kits (Jiancheng Biochemical, China). In brief, cell homogenates were mixed with the cell lysis buffer (Cell Signaling Technology, USA) containing complete protease inhibitor cocktails (Roche Biochemicals) to precipitate the protein. Then, all the processes were followed as the manufacturer's instructions described, and the samples were incubated at 95 °C for 40 min. The absorbance was measured at 532 nm to calculate the results, which were expressed as nmol per mL. Protein concentrations were measured by a BCA protein assay (Beyotime Biotechnology, Beijing, China). For superoxide dismutase (SOD) measurements, commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nangjing, Jiangsu, China) were used; the cell homogenates were prepared as described previously; and all procedures were carried out according to the manufacturer's instructions. The SOD activity was expressed as units/mg protein.

Western blotting

Cells were washed twice with ice-cold PBS, and then suspended in 100 μl of lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, protease inhibitor cocktail, 2 mM Na3VO4, and 10 mM NaF, pH 7.5). The protein concentration was determined using a BCA assay kit (Beyotime, Jiangsu, China). An equal amount of protein (20 μg) was loaded in each lane. Proteins were separated using sodium dodecylsulfate-polyacrylamide (SDS-PAGE) gel electrophoresis and electrically transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore). After blocking the membrane with 5% skim milk, target proteins were immunodetected using specific antibodies. After incubation with the secondary antibody, bands were visualized using an ECL plus kit (Pierce) and exposed to autoradiographic films according to the manufacturer's protocol. The intensities of bands were performed using Quantity One Software (provided by Bio-Rad, Hercules, CA, USA).

Statistical analysis

All quantitative data and experiments described in this study were repeated at least three times. Statistical analysis was performed using SPSS 17.0 software. Data were presented as mean ± standard error of mean (SEM), and statistical significance was determined using one-way analysis of variance (ANOVA) followed by multiple comparisons with Dunnett's test. A p-value < 0.05 was accepted as a statistically significant difference.

Results

Effect of curcumin on acrolein-induced neurotoxicity in HT22 cells

Results displayed that HT22 cells incubating with curcumin at the concentration of 5 μg/mL for 24 h exhibited the highest cell viability, which was statistically higher than vehicle group cells ($p < 0.05$, Fig. 1A); and, if the treatment time was prolonged to 48 h, the survival of HT22 cells was reduced, although without significant difference. When the level of curcumin was increased to 10 μg/mL, no significant increase of cell viability was observed; moreover, a marked decrease appeared if the curcumin level was increased to 25 μg/mL or more ($p < 0.05$, Fig. 1A). Therefore, 5 μg/mL curcumin was used in the following experiments to detect the protective functions of curcumin.

Data showed that cells that received curcumin exposure alone witnessed a significantly increased cell viability, whereas those exposed to acrolein alone experienced a sharp decrease when compared to vehicle cells ($p < 0.01$, Fig. 1B). However, the significant decrease of cell viability and the damage of cellular morphology induced by acrolein could be markedly reversed by curcumin ($p < 0.05$, Fig. 1B and C), which verified the protective effects of curcumin against acrolein-induced neurotoxicity *in vitro*.

Effect of curcumin on acrolein-induced apoptosis in HT22 cells

Data showed that curcumin significantly reversed the obvious apoptosis induced by acrolein exposure (Fig. 2).

Effect of curcumin on acrolein-induced oxidative damage in HT22 cells

It was found that curcumin significantly reversed the reduction of SOD level induced by acrolein ($p < 0.01$, Fig. 3C). Accordingly, the marked acroelin-induced increase of MDA and depletion of GSH were also obviously improved by curcumin, although without statistical significance (Fig. 3A and B). These results together provide evidence for the hypothesis of curcumin's capacity to relieve the acrolein-induced oxidative stress.

Effects of curcumin on acrolein-induced inhibition of the BDNF/TrkB signaling in HT22 cells

Results indicated that curcumin could increase the expression of TrkB alone and reverse the reduction of TrkB expression induced by acrolein dramatically ($p < 0.01$, Fig. 4B). The significant decrease

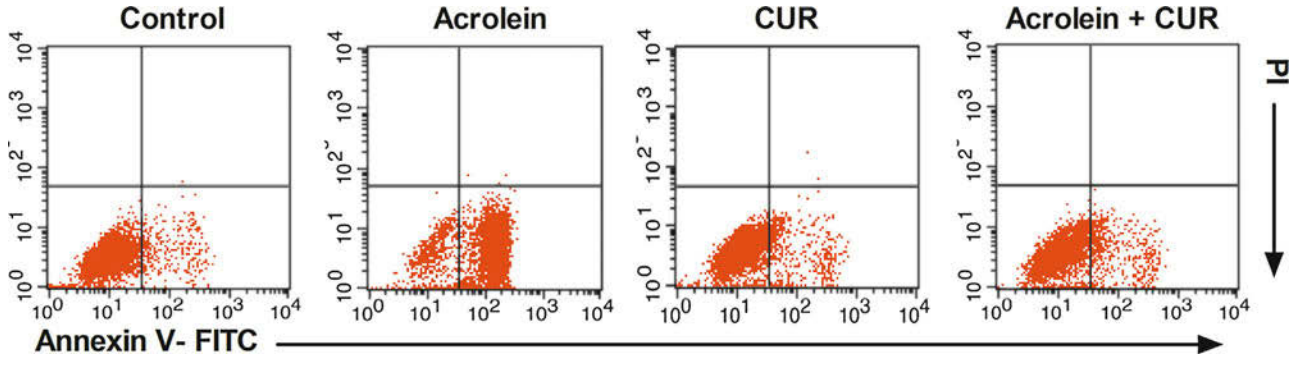

Fig. 2. Effects of curcumin on acrolein-induced apoptosis in HT22 cells. The apoptosis induced by 25 μM acrolein in HT22 cells was significantly decreased *via* incubating with 5 μg/mL curcumin (CUR) for 30 min earlier. Cell apoptosis was assessed based on flow cytometric analysis with an AnnexinV-FITC/PI assay kit.

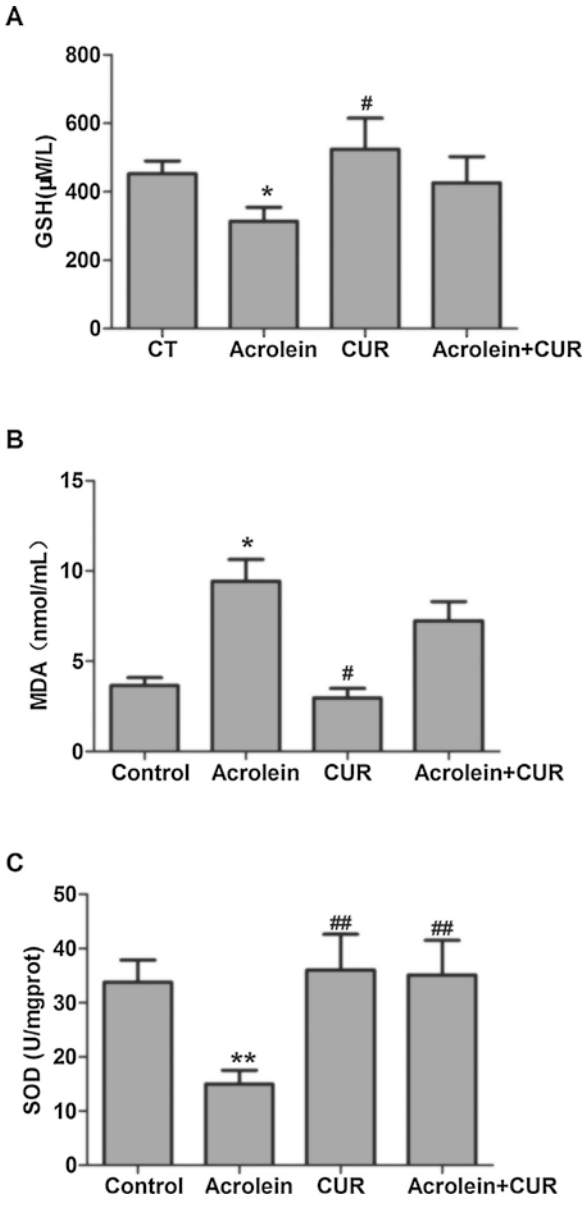

Fig. 3. Effects of curcumin on acrolein-induced oxidative stress in HT22 cells. Exposure of 25 μM acrolein for 24 h markedly increased the level of MDA and resulted in significant reductions in GSH and SOD contents in HT22 cells, which could be reversed by curcumin (CUR) supplement. Data are expressed as mean ± SEM. Intracellular levels of GSH (A), MDA (B) and SOD (C) in HT22 cells exposed to 25 μM acrolein for 24 h with or without pretreated with 5 μg/mL curcumin for 30 min. $^{*}p < 0.05$ and $^{**}p < 0.01$ *vs.* untreated control cells; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* model group acrolein-treated cells.

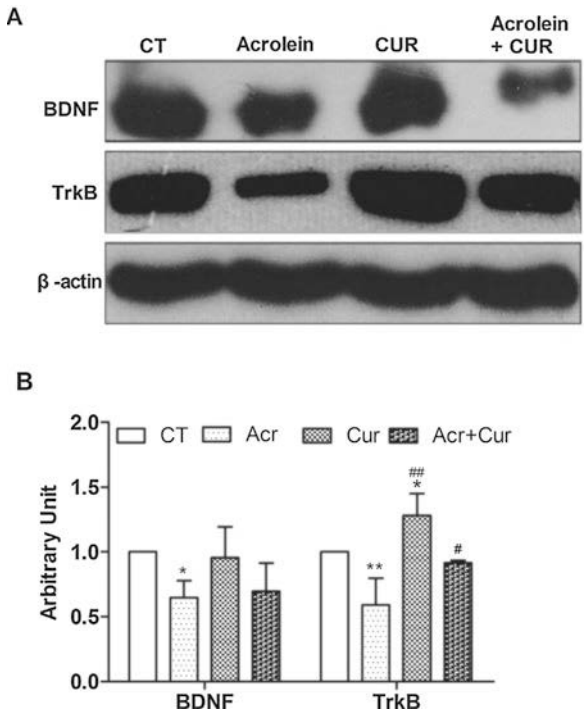

Fig. 4. Effects of curcumin on acrolein-induced inhibition of the BDNF/TrkB signaling in HT22 cells. Curcumin effectively restored the inhibited activity of TrkB induced by acrolein. Improvement of acrolein-induced down-regulation of BDNF level remained apparent with curcumin exposure, although without statistical significance. HT22 cells were treated with 25 μM acrolein (ACR) with or without pre-treating with 5 μg/mL curcumin (CUR) for 30 min. The expression levels of BDNF and TrkB were measured by western blotting. (A) Curcumin reversed acrolein-induced inhibition of the BDNF/TrkB signaling. β-actin was used as a protein-loading control. (B) Densitometric measurements of band intensity were performed using Quantity One Software. Data are expressed as mean ± SEM. $^{*}p < 0.05$ and $^{**}p < 0.01$ *vs.* untreated control cells; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* acrolein-treated cells without curcumin pretreated.

in BDNF caused by acrolein exposure was slightly restored by pre-incubation with curcumin, although the restored level was still lower than that at the normal baseline (Fig. 4A). These results indicated the capability of curcumin to relieve the acrolein-induced inhibition of the BDNF/TrkB signaling in HT22 cells.

Effects of curcumin on acrolein-induced alteration of key AD-associated proteins in HT22 cells

Data showed that A-disintegrin and metalloprotease (ADAM-10) decreased sharply, whereas amyloid precursor protein (APP), β-amyloid converting enzyme 1 (BACE-1), and receptor for advanced glycation end products (RAGE) increased dramatically after acrolein exposure for 24 h (Fig. 5A). All the alterations were nearly restored by curcumin (Fig. 5A, B), demonstrating that curcumin could adjust acrolein-induced changes of key AD-associated proteins in HT22 cells.

Discussion

In accordance with our previous study, AD-like pathology induced by chronic oral exposure to acrolein was observed again in HT22 cells [7,19]. Furthermore, curcumin was found to exert protective effects on acrolein-induced neurotoxicity with the potential mechanisms including mitigating oxidative stress, reversing the inhibition of the BDNF/TrkB signaling, and regulating enzymes involved in APP metabolism such as ADAM-10, BACE-1, and RAGE.

Despite the fact that AD has been studied for more than 100 years, the exact causes and pathogenic mechanisms remain to be clarified. As a result, the therapeutic options of AD are very limited. Currently available synthetic compounds applied in the treatment of neurodegenerative diseases, especially AD, failed to work as well as initially expected and often induce various side effects. Therefore, the natural compounds with potentially multiple targets of neuroprotective effects derived from plants, such as curcumin, vitamin C, and *Gingko biloba* and so on, have been investigated intensively in recent years [20]. It was hypothesized that the hydrophobicity of curcumin or the interactions between the keto or enol rings of curcumin and aromatic rings of Aβ dimers destabilized the attractions requisite for the formation of β-sheets in Aβ plaques [21]. In addition, curcumin also serves as the main

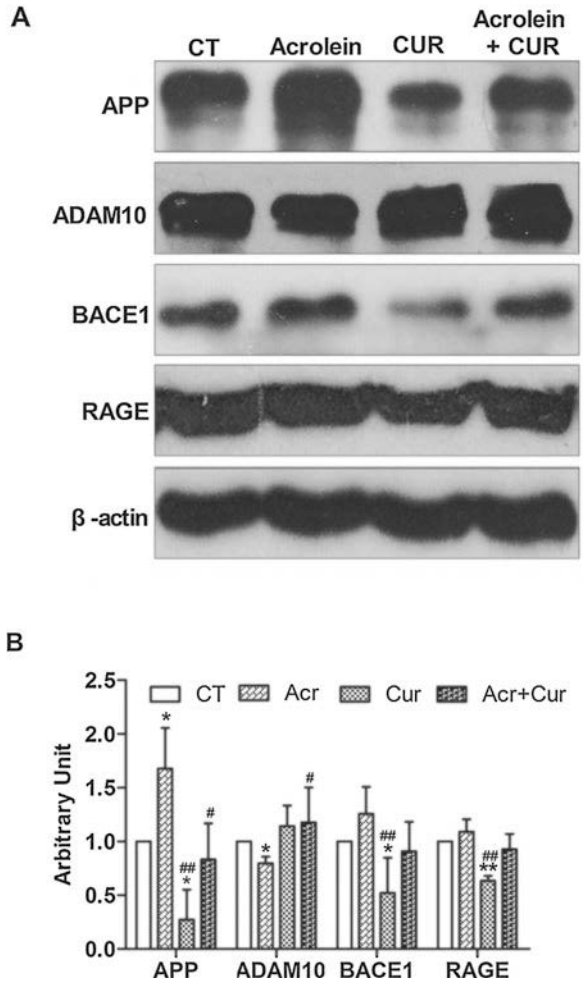

Fig. 5. Effects of curcumin on acrolein-induced AD pathologies-associated proteins'alteration in HT22 cells. Curcumin effectively reversed acrolein-induced reduction of ADAM-10 and increase of APP, BACE-1, and RAGE to the near-normal control levels. HT22 cells were treated with 25 μM acrolein (ACR) for 24 h with or without pre-treating with 5 μg/mL curcumin (CUR) for 30 min. Protein levels of APP, ADAM-10, BACE-1, and RAGE were measured by western blotting. (A) Curcumin reversed acrolein-induced harmful changes of APP, ADAM-10, BACE-1, and RAGE. β-actin was used as a protein-loading control. (B) Densitometric measurements of band intensity were performed using Quantity One Software. Data are expressed as mean ± SEM. $^{*}p < 0.05$ and $^{**}p < 0.01$ *vs.* untreated control cells; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* acrolein-treated cells without curcumin pretreated.

active ingredient of the curry spice, which is very popular in south and southeastern Asia. Interestingly, as the epidemiological studies reported, prevalence of AD is 4.4-fold lower in India compared with the United States population [22].

In this study, we explored the effects of curcumin on acrolein-induced neuro damages in HT22 cells and further revealed the potential mechanisms. When HT22 cells were pretreated with 5 μg/mL of curcumin for 30 min, the dramatically reduced cell viability induced by acrolein was restored to the control level and the damages of cellular morphology were also improved, which indicated the ability of curcumin to relieve acrolein-induced apoptosis in HT22 cells. Loss of neurons could lead to atrophy in the cerebral cortex and subcortical regions. Evidence indicated that curcumin and its analog could antagonize acrolein-induced cell death and mitochondrial dysfunction [23]. In our study, the marked apoptosis of HT22 cells induced by acrolein was reversed dramatically by curcumin. Acrolein-induced apoptosis was shown to be caspase-dependent in human neuroblastoma cells, and evidence also showed that curcumin could ameliorate the notably elevated pro-apoptotic protease enzymes caspase-3 and -9 induced by hydrogen peroxide exposure in SH-SY5Y neuronal cells [24,25]. Thus, the modulation of caspase may participate in the neuroprotection of curcumin against acrolein, which needs to be investigated further.

There was evidence shown that the oxidative stress induced by Aβ accumulation was one of the factors causing AD [26]. Curcumin has been proposed for the treatment of neurodegenerative diseases, as it promotes GSH formation [27,28]. It has been investigated for its antioxidant properties: directly through scavenging free radicals and indirectly through modulating the cytoprotective response such as upregulating the transcription of proteins involved in replenishing the antioxidant glutathione [28]. We also discovered that curcumin profoundly alleviated acrolein-induced oxidative stress through restoring the depletion of GSH and SOD, as well as reducing MDA contents.

The critical roles of BDNF, which is widely distributed in the hippocampus, cortex, and basal ganglion regions, in the survival of central and peripheral neurons as well as the promotion of synapse formation and the maintenance of synaptic plasticity through interactions with TrkB/P75 receptor have been verified before [29]. Curcumin may protect hippocampal and frontal neurons against stress-induced damages *via* upregulation of cyclic AMP response element binding protein (CREB) and BDNF/TrkB [30]. Recently, Motaghinejad et al. also found that curcumin conferred protective effects on nicotine-induced neurotoxicity *via* reactivation of the P-CREB/BDNF signaling pathway [31]. Likewise, our findings indicated that the inhibition of BDNF/TrkB signaling induced by acrolein could be dramatically reversed by curcumin. Thus, we may speculate that the mechanism behind the neuroprotection of curcumin against acrolein might partly lie in the reactivation of the BDNF/TrkB signaling. However, the reliability of this hypothesis should be reinforced by further exploring the neuroprotection of curcumin under the condition of blocking BDNF/TrkB signaling.

Curcumin targets two pathological markers of AD, Aβ and tau, to exert neuroprotective effects [28]. The inhibition of BACE-1 and subsequent suppression of Aβ production by curcumin has been verified in *vitro* [32]. Consistent with these evidences, curcumin was found to obviously downregulate the level of BACE-1 in our study. Moreover, an identifiable increase of BACE-1, although not significant, caused by acrolein, was reduced to a certain extent by curcumin, which implied the potential effect of curcumin in alleviating acrolein-induced neurotoxicity *via* modulation of β-secretase. Alternatively, α-secretase (ADAM family member, especially ADAM-10) acts as a protective member that facilitates the clearance of APP, to prevent Aβ formation [33]. Although the mechanisms underlying the neuroprotective effects of curcumin have been revealed gradually, investigations about its influence on α-secretase cleavage of βAPP are rare. Only Narasingappa RB et al. discovered that amino-acid conjugated curcumin promoted the constitutive α-secretase activity and increased ADAM-10 immunoreactivity [34]. In line with this research, our findings demonstrated that the significantly decreased level of ADAM-10 induced by acrolein could be profoundly restored by curcumin, which suggested curcumin's potential in regulating the activity of α-secretase against acrolein-induced neurotoxicity. The production of RAGE could be promoted by oxidative damages arising from Aβ deposition, whereas curcumin was reported to reduce the production and aggregation of Aβ as well as shift the Aβ aggregation pathway to the formation of nontoxic conformers such as soluble oligomers and prefibrillar aggregates [28,35]. Thus, curcumin was postulated to downregulate RAGE. Interestingly, our data showed that the RAGE level in the curcumin treated group was indeed significantly lower than that in the control group and the acrolein treated group. All the findings mentioned here proved

that curcumin can protect HT22 cells against acrolein-induced alterations of AD pathology-associated proteins.

As verified in this in *vitro* study, the mechanisms of curcumin protecting HT22 cells against to acrolein-induced neurotoxicity may be related to anti-oxidative stress, promoting activation of BDNF/TrkB signaling and restoring changes of AD-associated proteins. However, further studies conducted in a transgenic mouse model of AD and investigation of curcumin's effects on acrolein-induced neurotoxicity under the blocking of the BDNF/TrkB signaling should be done in the future to consolidate our evidence. Moreover, it is important to note that the low oral bioavailability of curcumin leads to discrepancy between *in vitro* and animal models findings and human trials outcomes and appears to hindered its ability to exert its effects in clinical studies [28]. Thus, future studies should also pay attention to ameliorating the bioavailability of curcumin so that the success of pre-clinical studies can be translated to clinical outcomes.

Conflict of interest

None.

Authors' contributions

Lan-Ying Shi and Jian Qin designed experiments and analyzed results. Lan-Ying Shi and Li Zhang performed experiments and wrote the manuscript. Hui-Li, Tao-Li Liu, Ji-Cai Lai and Zhi-Bing Wu helped with the manuscript revising and language editing.

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