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Curcumin facilitates the treatment of Alzheimer's disease via augmenting the function of antioxidant enzymes

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Abstract:

In China, Alzheimer's disease (AD) affects nearly 10 million people, or about one percent of the population, and how to treat AD has always been the focus of attention. This investigation examined the efficacy of curcumin in the treatment of AD by enhancing the activity of antioxidant enzymes. The findings will elucidate the involvement of the proposed therapeutic mechanisms, specifically the reduction in reactive oxygen species (ROS) and the augmentation of superoxide dismutase (SOD) activity. Unveiling the potential mechanism of action could yield novel insights into the application of curcumin for AD treatment, thereby aiding researchers in the advancement and utilization of curcumin.

Keywords:-Alzheimer's disease; curcumin; antioxidant enzymes

1. Introduction

Neurodegenerative disease, caused by the degradation of targeted neurons within the brain and spinal cord, can be fatal in severe conditions. Treatment of neurodegenerative disease has long been a great challenge, complicated by the intricate cerebral function [1]. The morbidity rate of this kind of disease is rising at an alarming speed all around the world, becoming a burden on the global public health system.

According to recent studies, the morbidity rate of diseases related to the central nervous system has surged by 59% in the past 30 years. And it has become one of the main contributors to global diseases. It is estimated that by the year 2021, the number of people plagued by neurological diseases will reach around 3.4 billion, accounting for 43.1% of the global population. The mortality rate of 2021 will be about 11 million all over the world [2]. With continuous advancement in molecular biology, neurobiology, and other subjects, researchers can now explore deep into pathological basis and develop more comprehensive treatment strategies.

Alzheimer's disease, short for AD, is a main form of dementia in aged people. Many symptoms of AD would occur, including impaired memory, deteriorated language, reduced physical abilities, and others. According to the latest data, by the year 2050, the number of people with dementia in Europe will be doubled, and that number around the world will be tripled [3]. Despite continuous advances in research tools and across-subject knowledge, the morbidity rate of AD continues to increase worldwide. Current treatment in this field focuses more on early diagnosis and symptom treatment, including imaging, cerebrospinal fluid tests, blood tests, and cognitive

assessments. For the moment, many interventions are available, but only acetylcholinesterase inhibitors (AChEI) and metyrapone are useful to treat AD at all stages [4].

Antioxidant enzymes, encompassing SOD, thioredoxin peroxidase (TPX), glutathione peroxidase (GSH-PX), and catalase (CAT), can mitigate the impact of free radicals and noxious substances on cellular structures, thereby preventing and ameliorating a spectrum of age-related disorders, including AD. For example, SOD functions as an efficacious serine protease within the body. It can selectively neutralize detrimental free radicals and facilitate the disproportionation reaction that converts superoxide radicals into hydrogen peroxide. A decline in the levels of SOD and CAT within the body can lead to the inability to curtail the oxidative damage inflicted by an overabundance of free radicals generated either by external aggressors or cellular senescence, culminating in the onset of various prevalent age-related maladies.

Curcumin, a primary yellow-colored compound derived from the rhizome of turmeric, serves as the principal active ingredient in turmeric, boasting properties such as anti-inflammatory [5], anti-cancer [6], and anti–oxidative [7]. It has the capacity to augment the activity of antioxidant enzymes, thereby substantially enhancing the body's antioxidant capacity. Moreover, curcumin is capable of neutralizing various ROS, including superoxide anion free radicals, hydroxyl free radicals, and nitrogen dioxide-free radicals. It also provides protective effects against a multitude of diseases, such as neuronal cell damage, hypoxia, and cancer, by inhibiting lipid peroxidation, bolstering endogenous antioxidant defense enzymes, and curtailing peroxynitrite formation [8]. Clinically, curcumin is regarded as possessing high safety profiles and broad-spectrum pathological activity [9]. Its substantial antioxidant properties indicate a significant potential in the management of diverse diseases triggered by oxidative stress, warranting further exploration to harness its potential for drug development. Nonetheless, despite the availability of particular techniques to enhance curcumin's utilization within the body, its overall bioavailability remains suboptimal, necessitating additional investigation into its application.

The current primary therapeutic agents for AD are AChEI and Memantine. However, these medicines are prone to elicit a spectrum of adverse effects, including nausea, vomiting, diarrhea, and muscle cramps [10]. Such occurrences detrimentally impact the quality of life and treatment compliance of patients. Moreover, these medications predominantly exert their actions on the cholinergic system, which is inadequate given the multifaceted nature of AD. Furthermore, the chronic financial implications of these medications can impose a substantial economic burden on patients and healthcare systems. Consequently, there is an imperative for the development of more holistic treatment modalities. Curcumin, which exhibits ease of extraction and affordability, could be considered as a viable and cost-effective therapeutic alternative.

SH-SY5Y is a commonly-seen human neuroblastoma cell line, playing an important role in researches on neurodegenerative diseases, neurotoxicity, and neuroprotective mechanisms. It is easy to be cultured and maintained in a laboratory, which facilitates its propagation in large scale. The first choice of its cultural medium is Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum.

Moreover, the SH-SY5Y cell line has apparent responses to a sort of pharmacological agents and neurotoxins, making it a great example to clarify the process of neurobiology and relevant pathologies [11].

This paper predicts that increased concentration and prolonged treatment time of curcumin can alleviate oxidative stress by increasing the activity of antioxidant enzymes and reducing ROS to hold Alzheimer's disease at bay. This study offers new insights into the molecular pathways of curcumin to treat AD, in which curcumin mainly regulates neuronal cells with the antioxidant axis. Moreover, the research tends to enhance the therapeutic efficacy of curcumin, thereby laying a foundational perspective for its potential utilization as a primary agent in the pharmacological management of AD.

2. Methods and Materials

Intracellular ROS content is detected by flow cytometry. The activities of SOD are caught by an enzyme detection kit. The cell morphology of neurons is determined by immunofluorescence staining. In this experiment, the resveratrol is regarded as the positive control, while DMSO/PBS is to be the negative control.

2.1 Construction of the AD Cell Model

The SH -SY5Y cells are cultivated in a complete Dulbecco's Modified Eagle Medium (DMEM) high glucose with 10% fetal bovine serum (FBS), 100 units / mL of penicillin, and 0.1 mg / mL of streptomycin [12]. The cells will be maintained in an incubator with a constant temperature of 37℃, 5% CO_2, and saturated humidity. The AD model group would be subjected to a specific concentration of 100 μM H_2O_2, while the control group will be assigned to be exposed to 10% dimethyl sulfoxide (DMSO). Both groups will be cultured continuously for a duration of 24 h.

2.2 Measurement of the ROS content

DCFH-DA (2',7'-dichlorofluorescein diacetate) fluorescent probe is utilized to assess the intracellular ROS con-

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tent. Cells at the logarithmic growth phase are harvested, and the cell concentration is adjusted to a range of (1~10) × 106 /ml before being seeded into a six-well culture plate. Prior to analysis, the cells are incubated with 10 μM DCFH-DA at 37℃ in the dark for 30 min. Subsequently, the cells are collected and the fluorescence intensity of the resulting dihydrodichlorofluorescein (DCF) is quantified using the NovoCyte flow cytometry (manufactured by ACEA, China) [13]. ROS content = fluorescence intensity of each group after treatment/fluorescence intensity of negative control group.

2.3 Evaluation of antioxidant enzyme activity

The cellular activities of SOD is assayed utilizing commercial kits in accordance with the manufacturer's guidelines ((Nanjing Jiancheng Bioengineering Institute, China) [13].

2.4 Determination of neuronal cell morphology

The morphology of SH-SY5Y cells is investigated via immunofluorescence staining techniques. Cells within each group are immobilized using 4% paraformaldehyde for a duration of 10 to 30 min. Following the incubation of the cells with a blocking solution containing BSA, specific primary antibodies are applied and allowed to bind for an interval of 1 to 2 h. Subsequently, PBS is utilized for cell washing. Upon completion of secondary antibody incubation with fluorescent labeling, such as FITC, TRITC, or Cy3, an additional washing step is conducted, followed by the application of a nuclear stain for nuclear labeling. The samples are sealed, and examination using a fluorescence microscope is performed to assess the cellular morphology and architecture based on the fluorescent signal [14]. Ultimately, image analysis software, such as ImageJ, is employed for the quantitative evaluation of the fluorescence intensity, enabling precise dimensions and morphometric analysis of the cells.

3. Positive and negative controls for the experiments

The experiment will apply the variable control method to reduce the potential effect caused by unknown variables. Three distinct detection methodologies are involved: fluorescence-based ROS assay, enzyme activity assays for SOD utilizing enzyme detection kits, and immunofluorescence techniques for the examination of cellular morphology.

FACS analysis indicates a descending trend in ROS levels across the duration of the experiment. The test subjects will be exposed to curcumin at concentrations of 1,10 and 100 μM, while the positive control group will receive 10 μM resveratrol. Cells were cultured for both 24, 36 and 48 h. The negative control group comprised the SH -SY5Y cell line cultured with only buffer, using the determined ROS levels as the baseline for comparison. Additionally, for the enzyme activity assessment, another set of buffer-only SH -SY5Y cells served as the negative control. The rate of percentage change in enzyme activity, determined via each measurement, was established as the standard baseline for comparison.

4. Statistical Analysis

SPSS 27.0 will be used for statistical analysis while ANOVA will be applied to examine the differences between groups. Each trial will be replicated for a minimum of 3 times in order to enhance data reliability. Statistical significance is set to be at $P < 0.05 (a=0.05)$.

5. Results

Table 1 The combination of possible results for test groups

Combination of possible results (CR)	Curcumin decreases ROS by FACS?	Curcumin increases SOD activity by kit?	Curcumin decreases neuron damage by fluorescence microscopy?	Supporting hypothesis?
CR1	+	+	+	Yes
CR2	+	+	-	Partially
CR3	+	-	+	Partially
CR4	-	+	+	Partially
CR5	-	-	+	Partially
CR6	-	+	-	Partially
CR7	+	-	-	Partially
CR8	-	-	-	No

The "+" sign indicates that the phenomenon in each column title, and the difference is significant compared to the negative control group. The "-" sign indicates that the phenomenon was not detected experimentally, which may be attributed to the contradiction between the observed results and the hypothesis, or the similarity with the negative control outcomes.

CR1: ROS fluorescence assay demonstrates a substantial reduction in ROS levels in SH-SY5Y cells treated with curcumin throughout the experimental period. According to the test with an enzyme activity detection kit, the activity of SOD treated with curcumin has no apparent increase. Observation under fluorescence microscopy shows the morphology of the SH-SY5Y cells, including both the cell size and dendritic architecture. Curcumin reduces the level of neuronal damage.

CR2: ROS fluorescence assay demonstrates a substantial reduction in ROS levels in SH-SY5Y cells treated with curcumin throughout the experimental period. According to the test with an enzyme activity detection kit, the activity of SOD treated with curcumin has been apparently increased. As can be observed under fluorescence microscopic that the morphology of SH-SY5Y cells, including cell size and dendritic structure, has changed. Curcumin cannot alleviate neuronal damage.

CR3: According to ROS fluorescence assay, the ROS concentration in SH-SY5Y cells treated with curcumin has apparently decreased during the experiment. According to the test with an enzyme activity detection kit, the activity of SOD treated with curcumin has no apparent increase. As can be observed under fluorescence microscopic the morphology of SH-SY5Y cells, including cell size and dendritic strauctured has changed. Curcumin can reduce the level of neuronal damage.

CR4: ROS fluorescence assay shows that ROS concentration in SH-SY5Y cells treated with curcumin has not significantly decreased during the whole experiment. According to the test with an enzyme activity detection kit, the activity of SOD treated with curcumin has an apparent increase. As can be observed under fluorescence microscopic, the morphology of SH-SY5Y cells, including cell size and dendritic structure, has changed. Curcumin can reduce the level of neuronal damage.

CR5: ROS fluorescence assay shows that ROS concentration in SH-SY5Y cells treated with curcumin has not significantly decreased during the whole experiment. According to the test with an enzyme activity detection kit, the activity of SOD treated with curcumin has no apparent increase. As can be observed under fluorescence microscopic, the morphology of SH-SY5Y cells, including cell size and dendritic structure, has changed. Curcumin can reduce the level of neuronal damage.

CR6: ROS fluorescence assay shows that ROS concentration in SH-SY5Y cells treated with curcumin has not significantly decreased during the whole experiment. According to the test with an enzyme activity detection kit, the activity of SOD treated with curcumin has an apparent increase. As can be observed under fluorescence microscopic, the morphology of SH-SY5Y cells, including cell size and dendritic structure, has changed. Curcumin cannot reduce the level of neuronal damage.

CR7: ROS fluorescence assay shows a significant reduction in ROS levels in SH-SY5Y cells treated with curcumin throughout the experiment. According to the test with an enzyme activity detection kit, the activity of SOD treated with curcumin has no apparent increase. Observation under fluorescence microscopy shows the morphology of the SH-SY5Y cells, including both the cell size and dendritic architecture. Curcumin doesn't mitigate neuronal damage.

CR8: ROS fluorescence detection shows that the ROS concentration in curcumin-treated SH-SY5Y cells doesn't decrease significantly during the whole experiment period. Analysis utilizing an enzyme activity detection kit reveals a non-significant enhancement in the activity of SOD in the curcumin-treated group. Observation under fluorescence microscopy shows the morphology of the SH-SY5Y cells, including both the cell size and dendritic architecture. Curcumin doesn't mitigate neuronal damage.

6. Possible results for the variables of concentration and treatment duration

Potential Factor 1: The intracellular ROS content and SOD activity exhibit a dependence on both concentration and duration. An escalation in the concentration of curcumin and the protraction of the treatment period could potentially lead to a reduction or augmentation in ROS levels, as well as a fluctuation in SOD activity.

Potential Factor 2: The intracellular ROS content and SOD activity are not solely dependent on concentration and time. Despite the escalation in curcumin concentration and extended treatment duration, the levels of ROS and SOD activity remain unchanged.

7. Discussion

CR1 shows that ROS levels decreased during the experiment, corroborating the antecedent hypothesis of ROS attenuation within the SH-SY5Y cell line. Analysis of SOD enzymatic activities indicates that treatment with curcumin potentiated these antioxidant defenses. Current research

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has substantiated that curcumin increases cellular capacity to scavenge ROS via modulation of such antioxidant enzymes such as SOD and CAT [12]. This finding aligns well with the empirical data, suggesting a promising enhancement in the therapeutic efficacy of the treatment regimen. The alterations in cellular morphology, induced by oxidative stress, are evaluated via immunofluorescence techniques. It also reveals a restoration of normal morphological characteristics in cancer cells, either partially or extensively, throughout the whole study. Such observations bolster the proposition that curcumin may exert therapeutic effects on AD through the aforementioned mechanisms. In general, it is recommended that additional experimental inquiries explore alternative potential mechanisms by which curcumin may contribute to AD therapy.

CR2 exhibits a parallel line of reasoning to CR1, in that there is a reduction in the ROS content and an augmentation in the SOD activity. Nevertheless, no recovery in the morphology of the damaged tumor cells is detected. This outcome can be attributed to two reasons: Firstly, despite a substantial decrease in the ROS content in comparison to the negative control group, the mitochondrial function and self-repair mechanisms of cancer cells are substantially compromised by the damage inflicted by hydrogen peroxide, thereby maintaining a state of oxidative stress. Secondly, curcumin facilitated the activity of SOD but did not markedly enhance the activity of other antioxidant enzymes, such as CAT, leading to an inadequate antioxidant effect. The first potential reason suggests that the ROS content is influenced by a multitude of factors, including cellular metabolism and repair mechanisms, which necessitates further investigation through a comparative analysis of cellular vitality. Regarding the second reason, it is advisable to allocate additional focus on the influence of various concentrations of curcumin on the activity of additional antioxidant enzymes, such as CAT and peroxidase (POD).

CR3 demonstrates a reduction in the ROS content, a decrease in the degree of damage to tumor cells, yet not a significant increase in the activity of SOD. Research indicates that curcumin confers cellular protection against oxidative stress through diverse mechanisms, including modulation of heme oxygenase [15], activation of the nuclear factor 2-related factor 2 (Nrf2)/antioxidant response element (ARE) pathway [16], and the NRF2-KEAPI signaling cascade [11]. The experimental findings corroborate the therapeutic efficacy of curcumin on AD to a certain degree, albeit with an inconspicuous enhancement of antioxidant enzyme activity observed. Further experiments may focus on other mechanisms of curcumin in the treatment of AD.

CR4 suggests that ROS levels don't decrease, and the healing of tumor cells is due to increased SOD activity through other pathways (MAPK/ERK, PI3K/Akt, etc.). One possible explanation is that the increased activity of SOD, in synergy with other neurotrophic factors (such as brain-derived neurotrophic factor (BDNF)), affects intracellular information transduction pathways and jointly promotes the repair of damaged neurons. The mechanism of antioxidant enzymes in the treatment of AD can be further studied through further experiments.

CR5 doesn't result in a reduction in ROS content nor an enhancement of SOD activity. However, the tumor cells demonstrate recovery. This outcome does not corroborate the proposed hypothesis, as the therapeutic efficacy against cancer does not align with the anticipated mechanism. Research has indicated that curcumin can ameliorate AD by diminishing the cerebrovascular accumulation of amyloid beta (Aβ) peptides [17]. It is plausible that curcumin possesses additional latent mechanisms in the treatment of AD, and further experimental investigation is required to delve into these potential avenues.

CR6 elucidates that curcumin markedly augments the activity of the SOD enzyme, yet it fails to mitigate the ROS content, and the severity of damage to tumor cells remains unabated. This suggests that the upsurge in SOD activity achieved in this experiment may not be adequate to neutralize the oxidizing free radicals within the cells. Consequently, it would be meritorious to broaden the concentration spectrum of curcumin treatment and extend the duration of treatment to validate these findings more comprehensively.

CR7 exhibits a parallel line of reasoning to CR3 in that there is a reduction in the ROS content; namely, curcumin attenuates cellular ROS levels via alternative pathways, including the augmentation of the activity of alternative antioxidant enzymes, such as CAT, rather than SOD. The insufficiency of curcumin in mitigating the detrimental extent of tumor cells might be attributed to the fact that, although ROS levels are substantially diminished compared to the negative control, the intracellular ROS concentration remains elevated relative to the positive control group, indicating that neuronal cells remain subjected to oxidative stress. Further investigation could elucidate the efficacy of other antioxidant enzymes, such as Catalase, and explore increased concentrations of curcumin treatment.

CR8 fails to corroborate the proposed hypothesis in its entirety, as no restoration of normal cellular morphology was observed, and the anticipated outcomes fail to materialize. One potential explanation is that the cancer cells were sufficiently compromised by the oxidative action

of H_2O_2 prior to treatment, resulting in their inactivation. The experimental maximum concentration of curcumin reaches 100 μM. Nevertheless, in vivo, a higher concentration of curcumin may be necessary to elicit enhanced antioxidant properties. Moreover, the treatment time may not be long enough for cancer cells to react, leading to no obvious changes in variables.

Future studies can try to explore the long-term effects of curcumin. In the future, extended experiments can be conducted with a extended concentration of curcumin. When more concentration degree are included, the experiment can be a more scientific and accurate one.

7.1 Discussion of the possible results with varied concentration and treatment duration

The first potential result suggests that ROS level and SOD activity inside cells can be affected with changes in the concentration and treatment time of curcumin. More study is needed to explore the most proper concentration and treatment time of curcumin.

The second potential result shows that ROS level and SOD activity inside cells are not fully dependent on the concentration and treatment time of curcumin. This might be caused by the inappropriate concentration or insufficient treatment time of curcumin. It is imperative that concentration degree and treatment time can be increased in further studies.

8. Conclusion

This paper has explored curcumin's treatment to AD by enhancing the activity of antioxidant enzymes. From these findings, the mechanism of proposed treatment might be clarified, particularly the reduction in ROS levels and the increase of SOD activity. Unveiling the potential mechanism could provide new insights into the use of curcumin in AD treatment, helping researchers to develop and employ curcumin well. This paper has explored all kinds of potential outcomes, and has provided suggestions for subsequent experiments. It is forecasted that future studies will mainly focus on the combined mechanisms of curcumin and its derivatives, and their applications in clinic practice.

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