Original Research

Monoamine oxidase inhibitors contribute to the treatment of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is the most common neurodegenerative disease, characterized by progressive memory loss and cognitive dysfunction. MAO inhibitors (MAOIs) are the main antagonists of MAO activity. At present, accumulated studies have shown that MAOIs can cause a significant increase in monoamines in the body, such as dopamine and serotonin, and the effects of these transmitters will be significantly enhanced, which can be used in the treatment of depression and Parkinson's disease. However, the mechanism of action of MAOIs on AD treatment needs to be further elucidated. In this study, we constructed AD animal models and cell models in vivo and in vitro to explore the role of MAOIs in the treatment of AD. We found that MAOIs can improve the cognitive impairment of AD model mice, reduce the number of apoptotic cells in the hippocampus and cortex of the brain and HT22 cells, and increase the proliferation level of HT22 cells. In addition, after treatment with MAOIs, the number of Nissl bodies in AD mice increased significantly, while amyloid plaques decreased significantly. We further found that MAOIs can reduce the expression of MAO-A, MAO-B, Aβ40 and p-Tau in mouse brain and HT22 cells, and increase the expression of 5-HT. In summary, we believe that MAOIs also have positive significance in the treatment of AD, and we suggest that the development of such drugs can be developed clinically.

Keywords: Alzheimer's disease; Monoamine oxidase inhibitors; Proliferation; Apoptosis; Cognitive disorder

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Introduction

Alzheimer's disease (AD) is a neuro-degenerative disease characterized by memory loss, cognitive impairment, and intellectual disability[1]. Worldwide, the prevalence of AD is increasing. It is estimated that by 2050, AD will affect more than 35 million people[2]. At present, AD is the most common neuro-degenerative disease in humans, leading to an increasing economic and clinical burden on modern society. The brain pathology of AD is characterized by neurofibrillary amyloid pla-ques and abnormal protein deposits, which are mainly caused by composed of amyloid-β peptide (Aβ) ($A\beta_{1\text{-}40}$ and $A\beta_{1\text{–}42}$) and intra-cellular insoluble hyperphosphorylated Tau protein (P-Tau) accumulation[3]. Studies have shown that AD starts in the brain area related to learning and memory, then spreads to the hippocampus, temporal cortex, frontal parietal cortex, and finally to the subcortical nu-cleus[4]. Currently, there are no diagnostic biomarkers for early detection of AD, and there are no effective drug treatments that delay or prevent the progression of AD. Therefore, further research is needed to explore more effective treatments for AD.

So far, the most widely accepted hypo-thesis on the pathogenesis of AD focuses on the gradual accumulation of Aβ[5, 6]. The destruction of this cascade has always been the main strategy for drug development[7]. About half of AD is Aβ-targeted drugs[8], and its treatment mechanism includes the regulation of Aβ production and removal of Aβ through immunotherapy[9, 10]. However, Aβ antibodies and β-amyloid secretase 1 inhibitors have repeatedly failed in clinical trials of AD, leading to changes in the current hypothesis of the etiology of this debilitating disease[11, 12]. Amine oxidases (MAOs) belong to the flavin monoamine oxidase family. It is an enzyme located in the outer mitochondrial membrane. It catalyzes the oxidative deamination of biological amines and heterogeneous amines, and plays an important role in the metabolism of neuro-active and vasoactive amines in the central nervous system and surrounding tissues. MAOs have always been a surprisingly stable source. They have a unique position in regulating the function of a variety of specific neurotransmitters, and are associated with various conditions, including mood dis-orders[13], anxiety and depression[14], schi-zophrenia[15], attention deficit[16], hyper-activity disorder[17], migraine[18] and sexual maturity[19]. MAOs have attracted people's attention to protein as a therapeutic target, and its application prospects in the treatment of a wide range of neurodegenerative diseases. This fin-ding suggests an alternative, Aβ-independent potential AD treatment strategy.

This study intends to explore the influ-ence of MAOIs on the development of AD in vivo and in vitro. It aims to provide basic experimental basis and treatment ideas for the treatment of AD.

Materials and Methods

Animal and cell treatment

Male APP/PS1 (APPswe and PSEN1dE9) transgenic mice and control littermates were purchased from Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, Yunnan, CN). This work was approved by The First Affiliated Hospital of Kunming Medical University. APP/PS1 mice were injected intravenously with 0.3 mg/kg MAOIs (Sigma, CN) or negative control, once every other day. for a total of one week. One week later, the mice were tested for clinical characteristics. After the mice were euthanized, pathological features were detected. The mice HT22 hippocampal neuron cell line was obtained from Shanghai Cell Bank of Chinese Academy

of Sciences (Shanghai, CN) and was Academy of Sciences (Shanghai, CN) and was maintained in DMEM culture medium (HyClone, CA, USA) containing 10% foetal bovine serum (FBS), 100 U/mL penicillin and 100 mg/mL streptomycin under 5% CO_2 at 37°C. To establish the AD model *in vitro*, HT22 were incubated with 10 μM Aβ1-42 for 24 h. The HT22 AD cell model was treated with 0.3 mg/kg MAOIs or negative control for 48 h.

Morris Water Maze (MWM) assay

The Morris water maze (MWM) was a deep pool (diameter: 120 cm, deep: 80 cm) filled with water made opaque with milk. The pool was divided into four quadrants with a platform submerged 1 cm beneath the surface in the target quadrant. Mice were trained for 5 consecutive days to find the platform (n = 12/group). A digital tracking device was used to measure movement. On day 7, the platform was removed, and the crossing time and distance in the target quadrant (%) were recorded.

Nissl's staining

Hippocampal and cortex sections were placed in 0.5% gelatin, attached to glass slides pretreated with gelatin and air-dried. Next, the sections were stained with Nissl staining solution (Beyotime, CN) for 15 min, dehydrated by gradient alcohol, differentiated with characteristic differential solution, and dehydrated again, followed by xylene permeabilization and sealing with neutral gum. Finally, the number of Nissl-positive neurons was counted under the light microscope.

Immunohistochemistry (IHC) assay

For animal tissue IHC analysis, formalin-fixed hippocampal and cortex tissue was embedded in paraffin and then cut into 35 μm sections using a freezing microtome (Leica, Germany). The sections were incubated with anti-Amyloid Precursor Protein antibody (1:500, Abcam, UK) overnight at 4°C, followed by incubation with a biotin-labelled secondary antibody. The signals were visualized using HRP-streptavidin with a 3,3′-diamino-benzidine (DAB) substrate. Images were acquired using a microscope.

Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling (TUNEL) assay

Hippocampal, cortex and HT22 cell slices were rinsed twice with PBS, incubated with 0.3% H_2O_2 for 15 min, rinsed again with PBS for 15 min, and then incubated with TUNEL staining solution (Beyotime, CN) according to the manufacturer's instructions. The number of TUNEL-labeled positive cells was measured by means of laser scanning confocal microscopy (Leica, Germany).

Cell Counting Kit-8 (CCK-8) assay

In short, HT22 cell s were seeded into96-well plates and incubated for 48 h. After that, the cells were incubated with 10 μL CCK8 (Amyjet, CN) for 2 h. Then, the absorbance109was measured to evaluate the viability of cells using a microplate reader (Mindray, CN) at 450 nm.

Western blotting assay

Protein was extracted from the hippocampus, cortex and HT22 cells using RIPA lysis buffer (Beyotime, CN). Equal amounts of protein were separated on 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were incubated with primary antibodies (Abcam, UK) overnight at 4 °C, and further incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Abcam, UK) for 1 h at 37 °C. Blots were quantified using ImageJ. Band density values were normalized to GAPDH.

Enzyme-linked immunosorbent (ELISA) assay

After induction or treatment, serum and the culture mediums of HT22 cells were collected into a centrifuge tube, and the supernatant was taken for test after centrifugation. The contents of $A\beta_{40}$ and $A\beta_{42}$ were measured by their corresponding ELISA Kits (MSK Bio, CN) according to the manufacture's instruction.

Statistical analysis

Data are shown as mean ± SD and analyzed with SPSS version 16.0 (SPSS Inc., Chicago, IL). The difference between two groups was analyzed with unpaired Student's t-test (two-tailed). The variance among multiple groups was analyzed with one-/two-way analysis of variance with/without repeated measures followed by the Bonferroni *post hoc* test. All experiments were performed three times, and $P < 0.05$ was considered statistically significant.

Results

Effects of MAOIs on cognitive impairment in APP/PS1 mice

As shown in the flow chart of the MWM experiment in Figure 1A, we first performed the Visible platform experiment for two days, followed by the Hidden platform experiment from the third, and finally, the Probe test from the 7th day. Fig. 1B is the water maze trajectory of the mouse on the second day. In the Visible platform, the Swimming speed of mice in the MAOIS group was significantly higher than that in the AD group, while the latency was opposite (Fig.1C). Interestingly, there was no significant difference between the data of the MAOIs and NC groups. In the Hidden platform, with the adaptation of the mice, the Escape latency time of the MAOIs group and the NC group was gradually shortened, while AD and DMSO group mice became stable (Fig. 1D). In the Probe test, the crossover number and time spent of the MAOIs group in the quadrant where the original Cue was located were significantly higher than those of the AD and DMSO groups, and there was no significant difference from the NC group (Fig. 1D). It is suggested that MAOIs are beneficial to the recovery of behavioral cognition in AD mice.

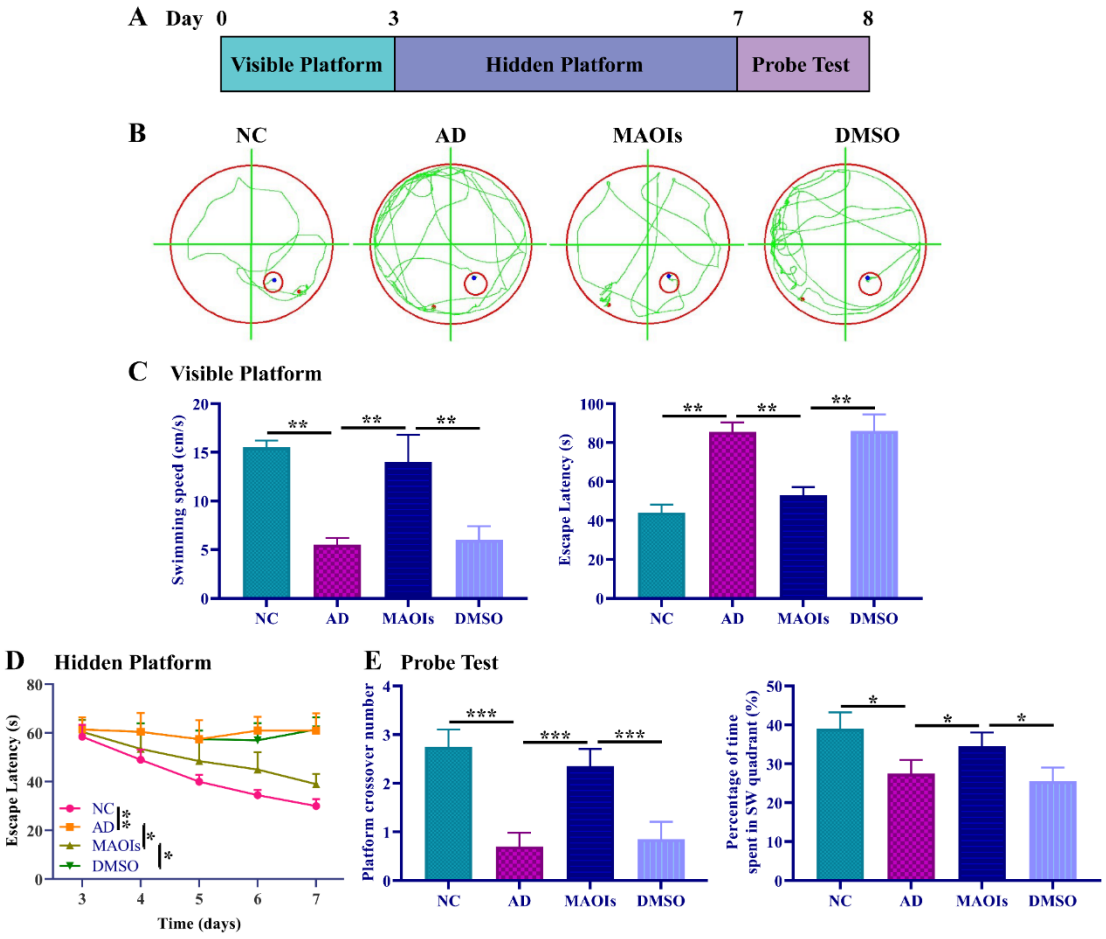

Figure 1 Effects of MAOIs on cognitive impairment in APP/PS1 mice. (a) Flowchart of the MWM experiment. (b) The trajectories of mice in each group in the visible platform experiment on the second day. (c) Statistical graphs of swimming speed and escape latency time of each group of mice in the visible platform experiment. (d) In the Hidden platform experiment, a statistical graph of the escape latency time of mice on day 3-7. (e) In the third stage of the probe test experiment, the mouse platform crossover number and time spend in swimming quadrant statistics. In all cases, Values are mean ± SD (n=6 for each group; *P<0.05, **P<0.01, ***P <0.001).

Effects of MAOIs on Aβ and Tau production and cell apoptosis in APP/PS1 mice brain

We further explored the effect of MAOIs on the pathology of mouse hippocampus and cortex. The results of Nissl staining in Fig. 1A shown that MAOIs could increase the number of Nissl bodies in the hippocampus and cortex of AD mice, and there was no significant difference from the NC group mice. In addition, compared with the AD and DMSO groups, the MAOIs group had clearly layered and regularly arranged hippocampal neurons, with complete cell membranes and nuclei, normal morphology, many clear Nissl bodies, and no obvious neurons. As expected, the IHC results of the Amyloid Precursor Protein showed that the number of amyloid plaques in the hippocampus and cortex of the AD group was significantly higher than that in the NC group, and MAOIs could overly reduce the number of amyloid plaques in the AD group (Fig. 2B). Furthermore, we used Elisa to detect the levels of Aβ40 and Aβ42 in mouse serum. The results showed that compared with the AD group, the levels of Aβ40 in the hippocampus and cortex serum of the MAOIs group were significantly reduced, but they had no effect on the levels of

Aβ42 (Fig. 2C). Western blotting also got consistent results, that MAOIs could significantly reduce the level of Tau phosphorylation in the hippo-campus and cortex of AD mice (Fig. 2D). In addition, we detected the level of apoptosis in the hippocampus and cortex by TUNEL staining. The results showed that the level of apoptosis in the brain of the AD group was significantly increased, and MAOIs could reduce the level of apoptosis in the hippo-campus and cortex of the AD group (Fig. 2E). It indicates that MAOIs can reduce the pathological damage in the hippocampus and cortex of AD mice, the expression of Aβ40 and p-Tau, and cell apoptosis.

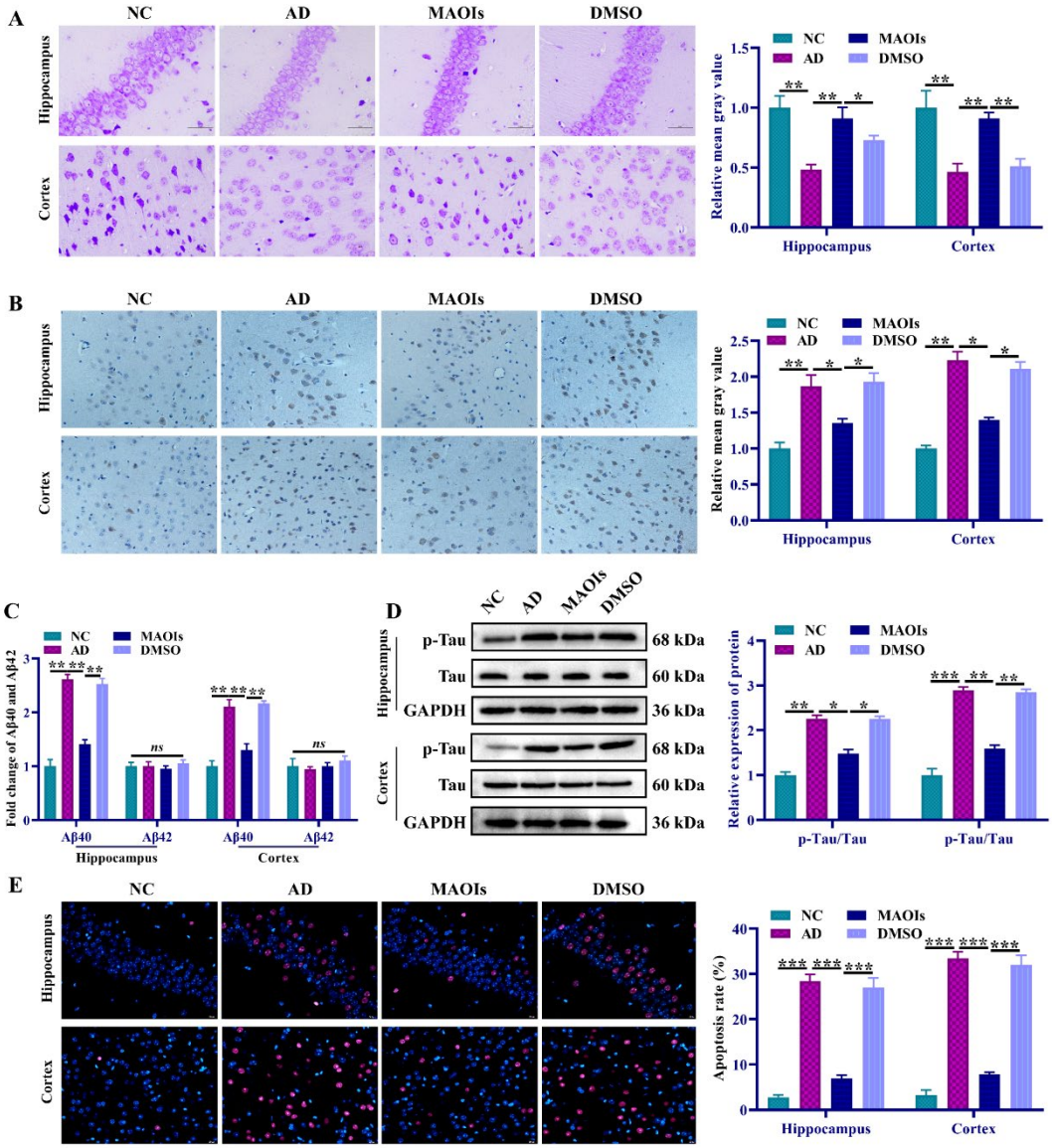

Figure 2 Effects of MAOIs on Aβ and Tau production and cell apoptosis in APP/PS1 mice brain. (a) Nissl staining was used to observe the number and status of Nissl bodies in the hippocampus and cortex of mice. (b) IHC staining was used to observe the accumulation of amyloid plaques in the hippocampus and cortex of each group of mice. (c) Elisa detected the levels of Aβ40 and Aβ42 in the serum of mice in each group. (d) Western blotting was used to detect the expression levels of p-Tau and background protein in the mouse hippocampus and cortex. (e) TUNEL staining showed the apoptosis of mouse hippocampus and cortex. In all cases, Values are mean ± SD (n=6 for each group; *P<0.05, **P<0.01, ***P <0.001).

Effect of MAOIs on 5-HT and MAO expression in APP/PS1 mice brain

5-HT and MAO play a pivotal role in the development of AD. Therefore, we tested the effects of MAOIs on 5-HT, MAO-A and MAO-B in mouse brain tissue. Western blot-ting results showed that compared with the NC group, the expression level of 5-HT in the brain of the AD group was significantly down-regulated, and MAOIs could antagonize the effect of AD on 5-HT (Fig. 3). For MAO, MAOIs could significantly down-regulate the expression levels of MAO-A and MAO-B proteins in AD brain tissue. It is suggested that MAOIs can reduce MAO-A and MAO-B in AD and increase the expression level of 5-HT. In addition, MAOIs may alleviate the development of AD in this way.

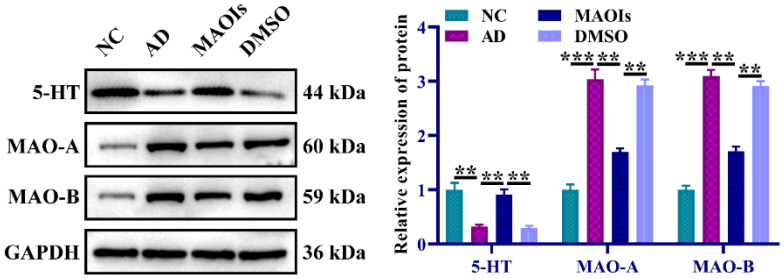

Figure 3 RAD21 is the target gene of miR-181a-3p. Western blotting was used to detect the expression levels of 5-HT, MAO-A and MAO-B in the brains of mice in each group. In all cases, Values are mean ± SD (n=6 for each group; **P<0.01, ***P <0.001).

Effect of MAOIs on the biological behavior of HT22 cells in vitro

Furthermore, we explored the effects of MAOIs on the proliferation, apoptosis, and AD-related protein expression *in vitro*. We processed HT22 cells with Aβ1-42 to construct an AD cell model, and then performed related tests. Western blotting results showed that the expression level of 5-HT in HT22 cells in the MAOIs group was significantly higher than that in the AD and DMSO groups, and there was no significant difference from the NC group. In contrast, MAOIs could down-regulate the expression levels of MAO-A and MAO-B in the HT22 cell model (Fig. 4A). CCK-8 results showed that MAOIs could rescue the down-regulation of HT22 cell proliferation caused by AD modeling (Fig. 4B). As expected, MAOIs could reduce the level of apoptosis in AD cell models (Fig. 4C). Subsequently, we tested the effect of MAOIs on the expression of Aβ and Tau in HT22 cells. Elisa results showed that MAOIs could significantly down-regulate the level of Aβ40 in the supernatant of HT22 cells, but had no significant effect on Aβ42 (Fig. 4D). In addition, Western blotting results showed that MAOIs could reduce the phosphorylation level of Tau in HT22 cells, but has no effect on the background level of Tau (Fig. 4E). In summary, we found that MAOIs could protect HT22 cells from damage caused by AD modeling, and inhibit the levels of Aβ40 and p-Tau in HT22 cells. These are most likely to be achieved by MAOIs by inhibiting the expression of MAO.

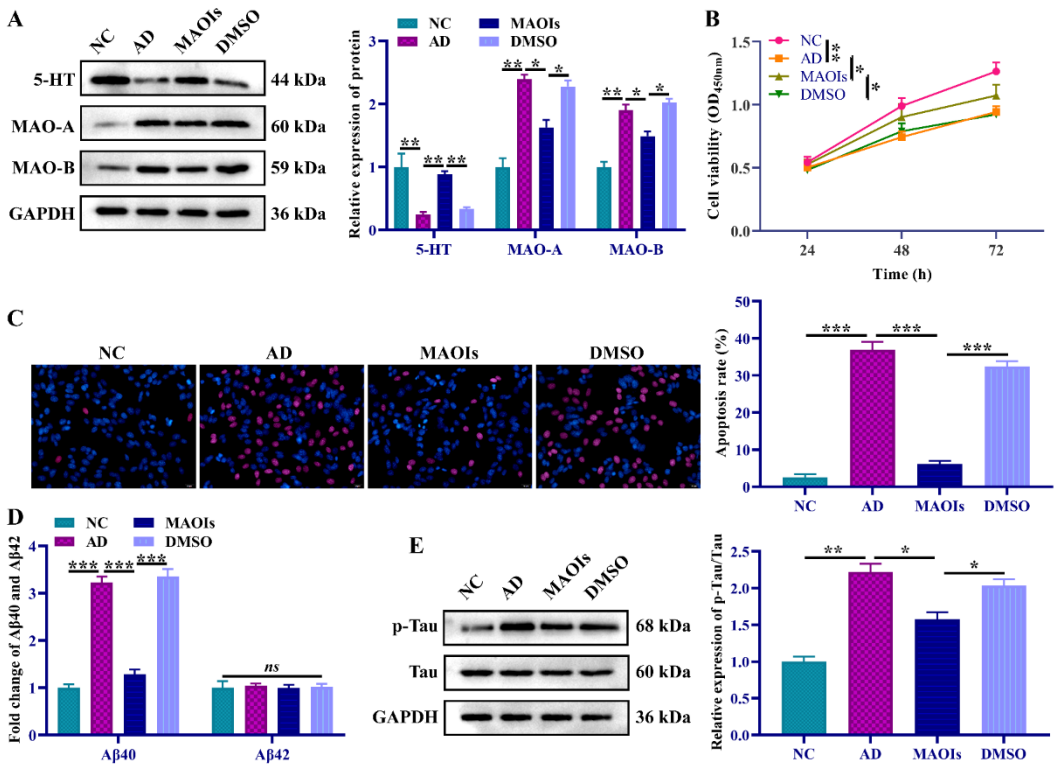

Figure 4 Effect of MAOIs on the biological behavior of HT22 cells. (a) Western blotting was performed to detect 5-HT, MAO-A and MAO-B expression. (b) CCK-8 kit exhibited the proliferation activity of HT22 cells in each group. (c) TUNEL staining showed the level of apoptosis of HT22 cells in each group. (d) Elisa detected the levels of Aβ40 and Aβ42 in the supernatant of HT22 cells in each group. (e) Western blotting displayed the expression levels of p-Tau and Tau in each group of cells. In all cases, Values are mean ± SD (n=3 for each group; *P<0.05, **P<0.01, ***P <0.001, ns means no significant difference).

Discussion

MAOIs have an interesting history: the original MAOIs were discovered in the early 1950s by doctors testing drugs used to treat tuberculosis[20]. Of the two hydrazine's studied (isoniazid and isoniazid), isoniazid proved to be the more effective antibiotic[21]. Isoniazid is less useful in this respect, but it is surprisingly valuable: it has mood-enhancing properties. Patients taking isoniazid showed greater vitality, more social activities, and expressed a desire to leave the hospital despite the severity of their illness[22]. This serendipitous discovery provided the basis for the first antidepressant drug[23]. Soon after, the pharmacological characteristics of isoniazid led to its identification as a MAO inhibitor. MAOIs have been used to treat depression since then and have been used to treat Parkinson's disease since the 1960s. However, the therapeutic effect of MAOIs in AD remains to be explored.

There are two isoforms of MAO, MAO-A and MAO-B. MAO-A is the main isoform in the intestinal tract[24]. It inactivates circulating catecholamines and dietary vaso-pressors and also plays a role in the breakdown of serotonin and catecholamines in the brain[25]. MAO-A inhibitors are used to treat psychiatric disorders[26]. However, their peripheral effects, when used in conjunction with tyramine or levodopa, cause the so-called "cheese effect", characterized by high blood pressure, palpitation, tachycardia, headache and

nausea[27]. When MAO-A inhibitors are combined with serotonin reuptake inhibitors, serotonin syndrome occurs, characterized by hallucinations, loss of coordination, tachycardia, changes in blood pressure, elevated body temperature, nausea, vomiting, and diarrhea[28]. These conditions can be life-threatening, and some MAO-BIS also occur, and at higher doses these lose selectivity to MAO-B and may also inhibit MAO-A[29]. However, such events have not been encountered in clinical trials[30].

The MAO-B subtype is dominant in the human brain and can decompose dopamine into 3, 4-dihydroxyphenylacetic acid and vanillic acid[31]. Mitochondrial dysfunction, especially the dysfunction of mitochondria-electron trans-port chain complex I and oxidative stress caused by dopamine-reactive metabolites play an important role in the pathogenesis of PD. MAO-B converts endogenous and exogenous dopamine enzymes into hydrogen peroxide. Therefore, oxidative stress and oxidative damage processes in PD are crucial. Aging and certain neurodegenerative diseases (such as AD and PD) demonstrate an association with 'elevated MAO-B levels, a phenomenon that is thought to be associated with increased oxidative stress occurring in these conditions[32]. Interestingly, MAO-B is an enzyme that converts 1-methyl-4-phenyl-1,2,3, 6-tetrahy-dropyridinium to an active neurotoxic metabolite, 1-methyl-4-phenylpyridiniumion, which can induce experimental or secondary Parkinson's disease [33]. MAO-B also activates other potential toxins, such as isoquinoline and β-carboline[34, 35]. MAOIS leads to increased dopaminergic activity in the striatum and may provide symptomatic benefits in dopamine deficiency by inhibiting dopamine break-down[36]. The increased amount of dopamine can be used to act on dopamine receptors, and the role of levodopa is also increased[37]. Inhibition of MAO-B reduces free radicals produced by dopamine oxidation and inhibits the conversion of MPTP to MPP + in animal models. Thus, MAOIs may have neuro-protective effects. However, this has not been confirmed in clinical trials[38, 39].

In conclusion, this study found that MAOIS improved the cognitive impairment of AD model mice. In addition, after MAOIS treatment, the number of Nissel bodies was significantly increased and amyloid plaques significantly decreased in AD mice. We further found that MAOIS decreased the expression of MAO-A, MAO-B, Aβ40 and p-Tau in mouse brain and HT22 cells, and increased the expression of 5-HT. We believe that MAOIs are also of positive significance in the treatment of AD. In clinical practice, we suggest to increase the study and experiment of MAOIS.

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