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Research of therapeutic basis of Astragalus P.E intervention based on the content of matrix metalloproteinase (MMP) protein in the serum of patients with Alzheimer's disease (AD)

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Abstract

Matrix metalloproteinases have been proven to be the target of Alzheimer's disease (AD) drugs. In this study, by monitoring the effects of different doses of Astragalus P.E on the serum level of matrix metalloproteinase (MMP) in patients with Alzheimer's disease. A cell model was established to explore the the protective effect and therapeutic basis of Astragalus P.E targeting matrix metalloproteinases on AD synapses. Using MTT to detect the effect of different doses of Astragalus P.E on the content of matrix metalloproteinase protein in the serum of patients with Alzheimer's disease; selecting the appropriate concentration and time as the modeling conditions of AD cells, transfecting PC12 cells by constructing the MMP promoter double luciferase reporter gene and Astragalus P.E was used as a treatment factor to observe the effect of Astragalus P.E on the transcription activity of MMP promoter. Observe the protective effect of Astragalus P.E on AD cell model and the effect on MMP expression. The AD cell model was transfected with MMP-siRNA to observe whether MMP is the key link in the effect of Astragalus P.E. 10 μM Astragalus P.E was used to induce PC12 cells for 24 h as the early AD cell model. Astragalus P.E can effectively inhibit the increase of transcription activity of MMP promoter induced by Astragalus P.E. Astragalus P.E can reduce the expression of MMP in PC12 cells induced by Astragalus P.E. At the same time, after transfection with MMP-siRNA plasmid, Astragalus P.E can significantly change the expression of MMP in PC12 cells induced by Astragalus P.E. Astragalus P.E targeting MMP has a protective effect on AD synapse damage, and it is a key link for the Astragalus P.E to exert medicinal effects.

Keywords: Alzheimer's disease; serum matrix metalloproteinase protein; Astragalus P.E; therapeutic basis.

Practical Application: Astragalus P.E targeting MMP has a protective effect on AD synapse damage.

1 Introduction

Alzheimer's disease (AD), as a common disease in the elderly, is an important type of senile dementia with the main features of progressive neuronal loss and synaptic damage (Zhang et al., 2018). In recent years, the research on the mechanism of AD has been continuously deepened, and its important focus is on the abnormality of tua of microtubule-related proteins, myloid peptides reaction, oxidative stress damage, cholinergic reduction, and inflammatory responses (Bahaeddin et al., 2018). The development of drugs related to pathological mechanisms has also been the main progress in recent years. There are 5 kinds of clinical drugs approved by the US FDA, mainly on cholinergic drugs (Py et al., 2014). In addition, supplementation of EPA and DHA during food intake plays a role in the protection from heart attacks, strokes, AD in the UK (Power et al., 2022; Wood et al., 2022). Tau protein hyperphosphorylation and Aβ erroneous protein aggregations are the main targets for drug development based on the pathological characteristics of AD, but most drugs are still in the research and development stage and have not yet entered clinical application. The clinical efficacy needs to be further tested (García-González et al., 2019). The traditional Chinese medicine has shown good research prospects in the treatment of AD. The research and development of AD traditional Chinese medicine based on the theory of traditional Chinese medicine is the main direction of future development. Astragalus P.E, is the dry root extract of leguminous plant Astragalus, the effective ingredients are astragaloside IV, astragalus polysaccharide (Shibata et al., 2005). Chinese medicine believes that astragalus is the nutrient for energy and antiperspiration, detoxing and drainaging abscess, inducing diuresis to alleviate edema. Research on central diseases has also been increasingly reported in recent years (Ishola & Adewole, 2019). Previous studies have shown that the Astragalus P.E can effectively protect AD nerve cells from damage. The molecular mechanism is that it can inhibit the apoptosis of nerve cells through the CaMKII/CREB/Bcl-2 pathway, while effectively inhibiting the expression of autophagy. Studies have shown that the inhibitory effect of Astragalus P.E on the abnormal aggregation of Aβ is not obvious, but it has a more significant effect on the expression of MMP (Lim et al., 2011). Memory loss, learning disabilities, etc. will happen in the onset of AD and directly damage the synapses. During the pathogenesis

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of AD, Aβ erroneous protein aggregation will also directly cause the overexpression of MMP, resulting in the abnormal activation of MMP and directly promoting the collapse of the growth cone and the retraction of neurites, which is an important molecular event in the inhibition of axonal regeneration and can also hinder the growth and regeneration of neuronal axons (Lim et al., 2011). In order to further study the mechanism of Astragalus P.E and understand whether it can target MMP to play a protective role in AD synapse damage, this study is expected to detect effect of the different doses of Astragalus P.E. on matrix metalloproteinase protein (MMP) in the serum of patients with Alzheimer's disease. Then build an AD cell model, combined with the construction of the MMP promoter reporter gene, MMP siRNA and specific expression, etc. Study whether Astragalus P.E targeting MMP has a protective effect on synapses from the MMP transcription level. It provides the basis for effective material science and also provides a new perspective for Chinese medicine on the research and development of AD.

2 Material and methods

2.1 Materials and instruments

Glutamine, penicillin (Gibco, USA); α-MEM medium, 0.25% trypsin, fetal bovine serum (FBS), LipofectamineTM 2000 (Sigma, USA); diphenyltetrazolium bromide (MTT) (Sigma, USA); dual luciferase activity detection kit, SOX4, β-actin antibody (Sigma, USA); inverted phase contrast microscope camera system (Hitachi S-4800, Japan); quantitative PCR reagents (Applied Biosystems, USA); Protein extraction kit (Bio-TEK Instruments, USA); scanning electron microscope (Nikon, Japan); Aβ protein fragment 25-35 (sigma), flow cytometry (Best Bio Shanghai); LipofectamineTM 2000 transfection reagent cationic lipid Carrier (KGI Bio Nanjing). AnnexinV-FITC Apoptosis Detection Kit (Invitrogen, USA), promega dual luciferase test kit, plasmids: PGL3-basic, PGL3-MMP promoters were constructed by Guangzhou Xinyi Biological Company. Astragalus P.E solution: Solubilized by 0.8% Tween 80, the concentration of the solution is 1 mg/mL.

2.2 Study case

25 serum samples were taken from patients with AD in our hospital from December 2018 to May 2019. At the same time, 25 serum samples of healthy volunteers were collected.

2.3 Cell culture

PC12 cells (from the Shanghai Branch of the Chinese Academy of Sciences Cell Bank) were cultured in an incubator and grown in RPMI 1640 medium, placed in the environment of 5% CO_2, 37 °C and 100% saturated humidity. When the single-layer cells grew to 80%-90% adhesion to the bottom then promoted decomposition with 0.25% trypsin and resuspended in culture medium containing 10% fetal bovine serum at inoculated density of 1 × 106/L. Regenerated in a 1:2 ratio for future use.

2.4 Experimental content

Experimental group: blank control group, model group (Astragalus P.E stimulating concentrations at 1 μM, 10 μM, 20 μM, 30 μM) Experimental steps: well-cultivated PC12 cells are planted in culture flasks at a density of 30%-40%. After culturing for 24 h, the culture flask was rinsed gently 2-3 times with D-hanks solution. The induction time was 7 days. Generally, the differentiation induction solution was replaced within 2-3 days.

2.5 MTT method to detect MMP content

Take PC12 cells in good condition after induced differentiation, remove the culture plate from the incubator, carefully aspirate the liquid from each well, shake for 10 min, add 100 μL/DMSO each well, then produce purple crystals, after crystal dissolving in DMSO, the optical density (OD) is measured with a microplate reader, and draw the MMP content curve at a wavelength of 490 nm.

2.6 The transcriptional activity of the MMP promoter in PC12

Grouping: PGL3-basic group, the experiment was set as MMP gene promoter group; after 12 h of transfection, the medium was changed, and cells were collected after 24 h for luciferase activity detection. Concentrations screening of Astragalus P.E stimulating MMP promoter transcription activity. The Astragalus P.E in the model group was diluted with complete medium to different concentrations. and the cells were collected for testing after 24 h of treatment. At the same time, tested the effect of Astragalus P.E on the transcription activity of MMP promoter.

2.7 Detection of mRNA and protein expression levels after transfection

After transfection for 24 h, TRIzol was used to extract RNA in each experimental group. Reverse transcription was performed using DNA as a template to obtain cDNA, and added PCR reaction solution [gene primer, red fluorescent dye (50 ×), dd H_20, Ex Taq]. The amplification is performed on the ABI7900 real-time PCR instrument. The reaction conditions: pre-denaturation at 95 °C for 1 min, 15 s at 95 °C, 30 s at 60 °C, 45 s at 72 °C, 40 cycles and pre-denaturation at 72 °C for 5 min. Calculate the relative expression ratio with the blank group.

48 h after transfection, total protein was extracted and subjected to protein quantification, electrophoresis, transfection and blocking. Added primary antibody and cultured for one night at 4 °C. After washing the membrane the next day, added secondary antibody, and then conducted band exposure processing. The digital imaging system processes and counts the bands. The ratio of the target protein band to the internal reference is used to indicate the relative expression of the obtained value.

2.8 Analysis of statistical results

Using the normal control group as a reference, collect the above research data and analyze with spss 20.0 statistical software. Univariate analysis of variance is used for comparison between multiple groups, and SNK test is used for pairwise comparison, $P < 0.05$ shows the data has statistical significance.

3 Results and discussion

3.1 Effect of different doses of Astragalus P.E on MMP content in serum of elderly AD patients

(Table 1) MTT data, the MMP content in the two groups is significantly different, and the relationship between MMP content and concentrations of Astragalus P.E each group is not obvious.By comparing the data of the two healthy control groups and the Astragalus P.E invention for AD patients, the effect of Astragalus P.E on MMP content was significantly different between the two groups. The MMP content of the healthy control group was higher than that of AD patients.

3.2 Transcription activity of MMP gene promoter in PC12 cells

(Figure 1) The transcriptional activity of the MMP gene promoter is 17 times than that of the PGL3-basic group. The comparison of the relative fluorescence values of the two groups has statistical significance, suggesting that the transfected MMP gene promoter can be expressed in PC12 cells.

3.3 The effect of different concentrations of Astragalus P.E on the transcriptional activity of MMP promoter

(Figure 2) Astragalus P.E 0.5 μM and 1 μM could not promote the increase of MMP transcription activity and did not reach the stimulating concentration; Astragalus P.E at 10 μM could stimulate the increase of MMP promoter transcription activity, and 20 μM could not stimulate the increase. 10 μM is the saturation concentration of cell transcription, so the action is most obvious ($P < 0.05$).

3.4 The effect of Astragalus P.E at different time on transcription activity of MMP promoter

(Figure 3) Compared with the control group, the Astragalus P.E fragment (10 μM) increased the transcription activity of the MMP promoter over time, the difference had statistical significance ($P < 0.05$), and the highest stimulation time was after 6 h. After that the transcription activity of the MMP promoter continually decreases, which is considered to be closely related to the transient transfection time.

Table 1. Effect of different doses of Astragalus P.E on MMP content in serum of elderly AD patients.

Astragalus P.E content	MMP content($\bar{x} \pm S$)	
	AD patients	Healthy control group
0 (control)	1.000 ± 0.044	1.002 ± 0.024
1 μM	0.494 ± 0.048	0.793 ± 0.024
10 μM	0.464 ± 0.056	0.692 ± 0.042
20 μM	0.474 ± 0.034	0.634 ± 0.042
30 μM	0.447 ± 0.047	0.626 ± 0.022

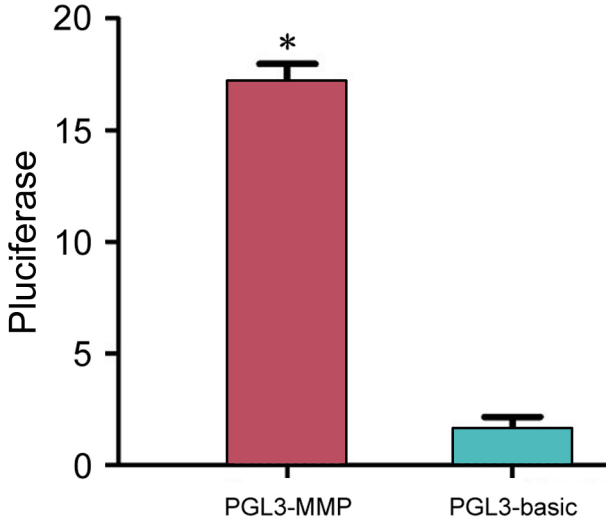

Figure 1. Transcription activity of MMP gene promoter in PC12 cells. *P<0.05.

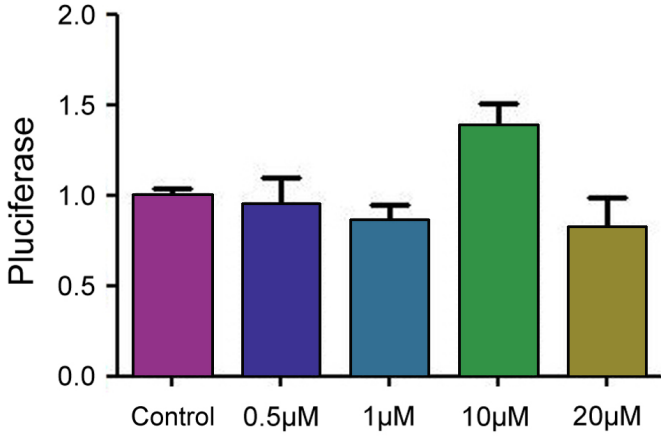

Figure 2. The effect of different concentrations of Astragalus P.E on the transcription activity of MMP promoter.

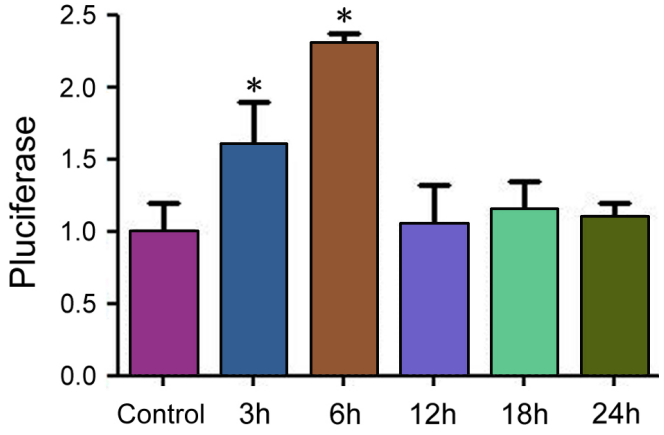

Figure 3. The different effect time of Astragalus P.E on the transcription activity of MMP promoter. Compared with control, *P<0.05.

3.5 Effect of Astragalus P.E on transcription activity of MMP promoter after stimulation of Astragalus P.E

(Figure 4) Low-dose (3 μg/mL) Astragalus P.E induces higher transcription activity of MMP promoter compared to high-dose (30 μg/mL) Astragalus P.E, and high-dose induction is similar to the inhibitory effect of MMP inhibitors, suggesting 30 μg/mL Astragalus P.E can effectively inhibit the transcription activity of MMP.

3.6 Astragalus P.E reduces the expression of MMP in injured PC12 cells

(Figure 5) Compared with the model group, the Astragalus P.E treatment group can effectively inhibit the expression of MMP, and the high-dose Astragalus P.E group (30 μg/mL) has a more obvious inhibitory effect, showing concentration-dependent ($P < 0.05$).

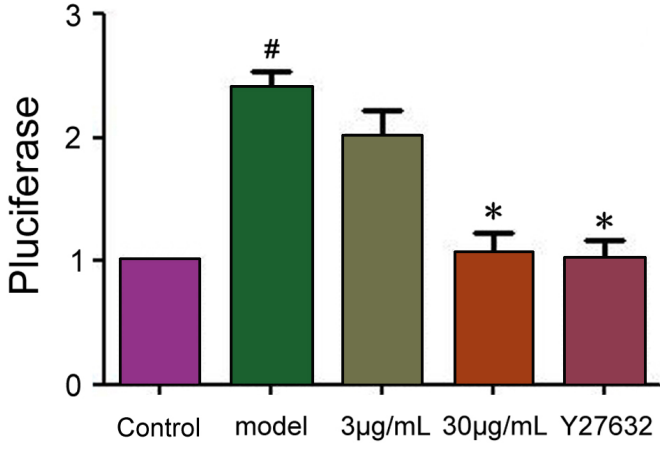

Figure 4. Astragalus P.E reduces MMP promoter transcription activity. Compared with control, #P<0.05; compared with model, *P<0.05.

In this experiment, we detected the effects of different doses of Astragalus P.E on the serum level of matrix metalloproteinase protein (MMP) in patients with Alzheimer's disease. The results showed that after stimulation of serum with Astragalus P.E, the MMP content of the healthy control group was higher than that of AD patients. It can be seen that MMP activity in serum cells of Alzheimer's patients is significantly reduced, but it is significantly increased after stimulation by Astragalus P.E. In order to further study the pharmacodynamic material basis of Astragalus P.E on AD patients, we establish an AD cell model and select PC12 cells derived from rat pheochromocytoma cells with neuroendocrine properties. The cell line has a long living time and stable passage. It has been confirmed that it can be used as an AD cell model to prove the protective effect of Astragalus P.E on nerve cells (Baig et al., 2008; Baig et al., 2006).

Based on the current research of the pathogenesis of AD, the Aβ cascade theory is the most influential. The study believes that Aβ is aggregated in a large number in AD patients. It is the key link and an important cause of the incidence (Baranger et al., 2016b). High-concentration of Aβ, β-strands and fiber formation are the basis of neurotoxicity (Baranger et al., 2016a). In this experiment, Aβ25-35 was used as the cell modeling reagent. In order to confirm the appropriate AD cell model and to study the protective effect of Astragalus P.E on synapses, we reduced the content of MMP to suppress the occurrence of apoptosis in cell experiments. Astragalus P.E has a protective effect on synaptic damage caused by Aβ. In order to detect the protective effect of Astragalus P.E on synapses and the relation with the expression of MMP, we constructed the MMP promoter reporter gene plasmid and transfected AD cell models. The experimental results showed that the Astragalus P.E can effectively reduce the increasing transcription activity of MMP promoter induced by Aβ25-35, further suggesting the protective effect of Astragalus P.E on the synapses of AD cell model. MMP content has a cascade effect with Aβ25-35, which can induce the transcription activity of the MMP promoter, but has a certain selectivity to

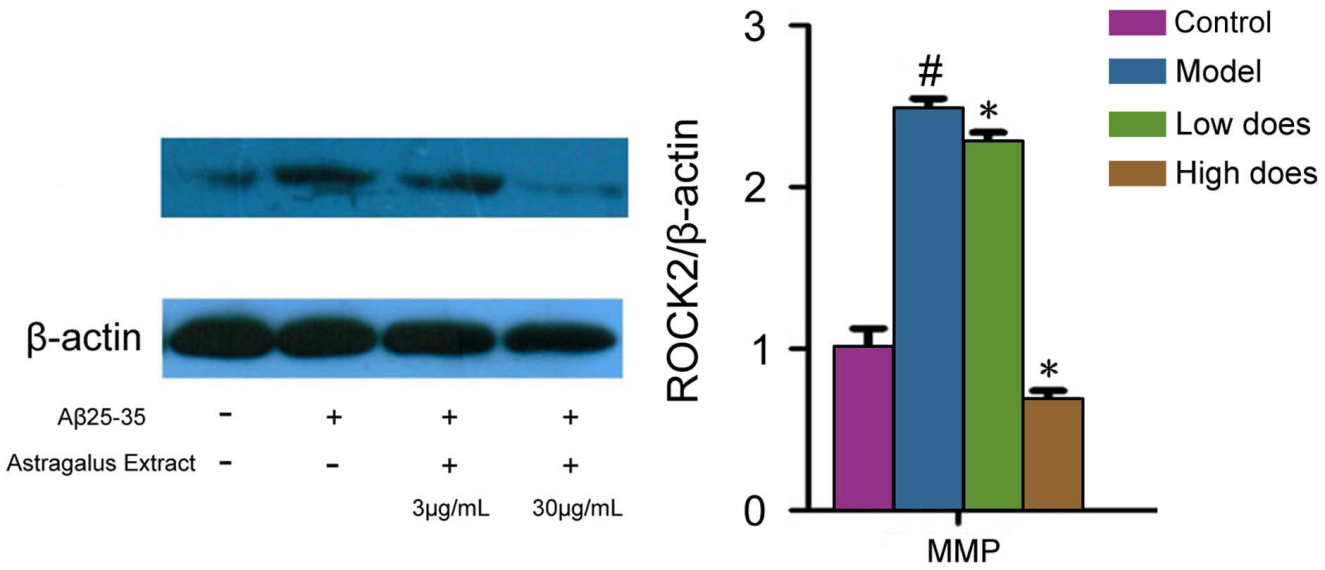

Figure 5. Astragalus P.E reduces the expression of MMP in injured PC12 cells. Compared with control, #P<0.05; compared with model, *P<0.05.

the concentration of Astragalus P.E. 10 μM Aβ25-35 is selected to act on PC12 cells and primary hippocampal neurons. Cells were induced into AD cell model after 24 h. In the AD cell model, it can be seen that the expression of MMP is significantly increased, and the Astragalus P.E can reduce the expression level of MMP, which has a certain protection effect on the synapses of AD cell model, and this protective effect is negative to the expression of MMP protein. In order to explore whether MMP is involved in the protective effect of Astragalus P.E on nerve synapses and whether it is a central link, we further verify it in AD cell models through extrinsic factor and infecting MMP genes. Interfering RNA (RNAi), as a double-linked RNA, can directly bind to mRNA and undergo specific intracellular degradation, thereby silence the target gene. With the confirmation of the corresponding phenotype of the target gene, RNAi can play a role in inhibiting the mRNA reaction, so that silence the gene after transcription (Kaminari et al., 2018).

Among them, siRNA is a guiding element for the degradation of RNA (mRNA) and plays an important role in the RNA silencing pathway (Kaminari et al., 2017; Shastry & Tyagi, 2003). siRNA is a necessary factor for RNAi to exert its effect and an intermediate product in the RNAi pathway (Yoshiyama et al., 2000). In this study, we explored the post-transcriptional level of MMP gene, transfected MMP-siRNA into AD cells, and established a positive control group to further understand the relationship between Astragalus P.E and MMP. After successfully silencing MMP, we analyzed the MMP expression in injured PC12 cells. The result showed that the cell damage was more serious after expression of MMP, and the damage could be significantly reduced after MMP treatment,which suggests that there is a close relation between the protective effect of Astragalus P.E on AD cell model and MMP. Studies have shown (Dingyu et al., 2011; Yoshiyama et al., 2000) that abnormally increasing Aβ protein in AD can significantly increase the expression of MMP, mainly manifested as the inhibition of brain synapses, MMP is also one of the important substances that increases Aβ (Abe et al., 2020; Conti et al., 2019). In this experiment, the results show that the Astragalus P.E can effectively inhibit the expression of MMP to protect the synapse from damage, providing a new perspective for the clinical research of AD and a theoretical basis for Chinese medicine for the effective treatment of AD.

4 Conclusion

Astragalus P.E targeting MMP has a protective effect on PC12 cell damage induced by Aβ25-35. Astragalus P.E may have a protective effect on injured synaptic through the regulation of MMP. The above research provide therapeutic basis for Astragalus P.E intervention in AD.

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