Effects of Donepezil and Galantamine on ET-1, CGRP, CER, BDNF and miR-132 Levels in the Treatment of Alzheimer's Disease

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Abstract: To investigate the effects of donepezil and galantamine on the levels of endothelin-1 (ET-1), calcitonin gene-related peptide (CGRP), ceruloplasmin (CER), brain-derived neurotrophic factor (BDNF) and miR-132 in the treatment of Alzheimer's disease (AD). **Methods** A total of 128 patients with mild to moderate AD admitted to our hospital from February 2019 to February 2021 were selected as the study subjects. According to the random number table method, they were divided into donepezil group (65 cases) and galantamine group (63 cases), and received donepezil and galantamine treatment, respectively, for 3 months. Before and after treatment, the cognitive function and symptoms of patients were assessed by the Mini Mental State Examination (MMSE), Alzheimer's disease assessment scale (ADAS-cog), the AD Subscale-Activities of Daily Living Scale (ADCS-ADL) and the Neuropsychiatric Inventory (NPI). The levels of ET-1, CGRP, CER, BDNF and miR-132 were determined to evaluate the safety of the treatment. **Results** ① After treatment, MMSE score in both groups increased, ADAS-cog score and NPI score decreased ($P<0.05$), and there was no statistically significant difference between the two groups ($P>0.05$). ② After treatment, the serum ET levels in both groups decreased, while the levels of CGRP, CER, BDNF and miR-132 increased ($P<0.05$). The levels of BDNF and miR-132 in donepezil group were higher than those in galantamine group ($P<0.05$). ③ The overall incidence of adverse reactions in donepezil group and galantamine group was 16.92% and 15.87%, with no significant difference between the two groups ($P>0.05$). **Conclusion** Donepezil and galantamine have good efficacy and safety in the treatment of mild to moderate AD, but donepezil may have some advantages in improving neurotrophic molecules.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease with insidious onset and progressive development. Clinically, it is mainly characterized by memory loss, aphasia, spatial function impairment, executive dysfunction and personality change. Data show that there are about 3 ~ 4 million AD patients in China. It has become a common cause of cognitive impairment in the elderly. At present, no specific drugs can reverse the progression of AD, so early diagnosis and intervention have become the focus and difficulty of clinical research. Donepezil and galantamine are cholinesterase inhibitors and first-line drugs in the treatment of AD, which can improve cognitive function mainly by slowing down the degradation of acetylcholine. Some studies have shown that the two drugs have certain effects on slowing down the progression of AD, but different studies have included different disease courses and disease degrees, and there are no consistent conclusions on the merits of the two drugs. Currently, there is no unified standard for the evaluation of cognitive function in AD patients, but there are some limitations in evaluating the effect of drug therapy from the perspective of cognitive function improvement. The analysis combined with blood markers is helpful to more intuitively analyze the effect of drug therapy and possible mechanism of action. It is commonly thought that vascular endothelial active, oxidative stress, neurons damage process is

closely related to cognitive function damage. Endothelin-1 (ET-1), calcitonin gene related peptide (CGRP) is a molecule associated with vascular endothelial activity. Ceruloplasmin (CER), brain-derived neuro-trophic factor (BDNF) are the molecules involved in nerve repair. The expression of miR-132 is closely related to the expression of nerve injury repair molecules. Analyzing the level of these markers may clarify the effect of drug therapy and the possible mechanism from the molecular perspective, and provide more ideas for clinical practice. Based on this, this study evaluated the therapeutic effects of donepezil and galantamine on AD patients admitted to our hospital, and evaluated their specific effects from the changes of serum marker levels, providing some reference for the selection and clinical application of the two drugs. The report is as follows.

2. Data and Methods

2.1 The general data

Patients with mild to moderate AD admitted to our hospital from February 2019 to February 2021 were selected as subjects. Inclusion criteria: age <80 years; Conforming to the diagnostic criteria of Chinese Classification and Diagnostic Criteria for Mental Disorders, 3rd Edition; Mini-mental State Examination (MMSE) evaluated as mild and moderate, with scores of 10-26; Hamilton Depression Scale score <17; The course of disease is 6 months or more; The patients were informed of the study and signed the consent form. Exclusion criteria: organic lesions resulting in cognitive dysfunction; Other types of dementia; Those who are assessed as having severe AD; Complicated with serious organic lesions and malignant tumors; Patients with impairment of consciousness, hearing and language ability; Having a history of mental illness; Those who used anti-dementia drugs before enrollment. Exclusion criteria: poor compliance, failure to complete treatment due to drug intolerance, and follow-up.

A total of 128 patients meeting the above criteria were divided into donepezil group (65 cases) and galantamine group (63 cases) according to the random number table method. There were no significant differences in gender, age, course of disease, MMSE score and education level between the two groups (P>0.05). See Table 1 for details.

Table.1. The comparison of general data of patients

group	case	Gender (male %)	Age (year)	Course (month)	MMSE (score)	education level (case %)		
						primary	high school	college or higher
donepezil	65	35 (53.82)	66.85±7.48	8.52±1.96	19.78±3.52	15 (23.08)	40 (61.54)	10 (15.38)
galantamine	63	33 (52.38)	67.12±6.98	9.01±2.12	20.01±3.01	13 (20.63)	39 (61.90)	11 (17.46)
χ^2/t value		0.028	0.211	1.359	0.397	0.175		
P value		0.868	0.833	0.177	0.682	0.918		

2.2 Methods

Donepezil group was given Donepezil hydrochloride tablets before bedtime (National Drug Approval H20070181, specification 5mg, China Eisai Pharmaceutical Co., LTD.). 5mg in the first 4 weeks (10mg after 4 weeks; galantamine group was given galantamine hydrobromide tablets (National drug approval H20061113, Xi 'an Janssen Pharmaceutical Co., LTD., specification 4mg), 2mg in the first week, 4mg in the second to fourth week, and 6mg in the fourth week. The treatment course of both groups was 3 months.

2.3 Observation Indicators

Cognitive function evaluation: MMSE and Alzheimer's disease assessment scale-cognitive plus (ADAS-cog) were used before and after treatment. MMSE is divided into orientation, memory, attention, computation, recall and language skills, with a total score of 30; The ADAS-cog score was divided into memory, attention, reasoning ability, orientation, application ability and language ability, with a total score of 70; The higher the score, the more impaired the cognitive function.

Daily living assessment: Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL) was used for evaluation, with a total of 20 items. The higher the score, the worse the ability to live independently.

Evaluation of neuropsychiatric symptoms: The Neuropsychiatric Inventory (NPI) was used to evaluate the 12 common psychiatric and behavioral symptoms of dementia. According to the frequency and severity of symptoms, each symptom was scored 0-12 points, with a total score of 144 points, and the average score of each item was used for analysis.

Biochemical indicators: Fasting venous blood was collected in the morning before and after treatment, and divided into 2 parts. One part of serum was centrifuged to detect ET, CGRP, CER and BDNF. Total RNA was extracted from the other part, and the expression of miR-132 was determined by quantitative PCR. Specific detection methods: (1) Serum indicators: Whole blood was taken and supernatant was separated by centrifugation at 2000r/min. The expression level of CER was detected by chemiluminescence method. The detection instrument was ACCESS automatic chemiluminescence immunoanalyzer produced by American Backman Company. The serum ET, CGRP and BDNF levels were detected by ELISA. The ET kit was purchased from Shanghai Xingyi Biotechnology Co., LTD., the CGRP kit was purchased from Shanghai Keshun Biotechnology Co., LTD., and the kit was purchased from Shanghai Enzyme Linked Biotechnology Co., LTD., and the RT-6000 marker from Shenzhen Leidu Co., LTD. All tests were performed by the same laboratory physician. (2) miR-132 expression detection: Whole blood was collected, total RNA was extracted using The Beijing Tiangen Biological Human Whole blood RNA Extraction Kit, and cDNA was synthesized using the Takara RR036A PrimeScript RT Master Mix reverse transcription kit. With cDNA as the template, real-time fluorescence quantitative PCR was used to detect its expression. Forward primers: 5'- ACGTCGTGTAGCTTATAAGACTG-3', reverse primer: 5'-ACTGCTTGTTCTACACACTCTG-3', the internal parameter is U6, the PCR detector automatically collects the amplified fluorescence signal and records the cycle number (Ct) when reaching the threshold value, ΔCt= target amplification Ct value - internal parameter Ct value, and the expression level of the target product is represented by $2^{-\Delta\Delta Ct}$.

Safety evaluation: assessment was conducted once a month during the medication period, including vital signs and adverse drug reactions. Liver and kidney function examination and electrocardiogram examination were performed after the treatment.

2.4 Statistical analysis

SPSS19.0 was used for data processing and statistical analysis. The count data were expressed as frequency and rate, and the inter-group comparison was performed by χ^2 test or *Fisher* exact test. The measurement data were tested in accordance with normal distribution, expressed as mean ± standard deviation ($\bar{x} \pm s$), and the inter-group comparison was performed by independent sample *t* test.

3. Results

3.1 Cognitive function of the two groups was evaluated before and after treatment

There was no statistically significant difference in the scores of MMSE scale and ADAS-cog scale between the two groups before treatment (P>0.05), but the scores of MMSE scale increased

and ADAS-cog scale decreased significantly after treatment (P<0.05), and there was no statistically significant difference between the two groups (P>0.05). See Table 2 for details.

Table.2. Cognitive function evaluation of the two groups before and after treatment ($\bar{x} \pm s$, points)

group	case	MMSE		ADAS-cog	
		before	after	before	after
donepezil	65	19.78±3.52	22.12±2.63*	20.55±2.96	17.72±2.22*
galantamine	63	20.01±3.01	22.33±3.41*	20.48±3.11	18.39±2.10*
t value		0.397	0.391	0.130	1.753
P value		0.682	0.697	0.895	0.082

Note: Compared with before treatment, *P<0.05

3.2 Evaluation of dementia scale before and after treatment

NPI score decreased in both groups after treatment, but ADCS-ADL score did not change significantly; There was no statistically significant difference in ADCS-ADL score and NPI score between the two groups before and after treatment (P>0.05). See Table 3 for details.

Table.3. Evaluation of daily living ability of the two groups before and after treatment ($\bar{x} \pm s$, points)

group	case	ADCS-ADL		NPI	
		before	after	before	after
donepezil	65	50.88±18.52	51.89±15.26	8.12±1.52	6.22±1.35*
galantamine	63	50.78±16.28	51.17±14.82	7.89±1.63	6.38±1.29*
t value		0.033	0.271	0.825	0.685
P value		0.974	0.787	0.410	0.495

Note: Compared with before treatment, *P<0.05

3.3 Changes of vasoactive regulatory factor levels in both groups before and after treatment

After treatment, serum ET level decreased and CGRP level increased in the two groups (P<0.05), but there was no statistically significant difference between the two groups (P>0.05). See Table 4 for details.

Table.4. Changes in the levels of vasoactive regulatory factors in the two groups before and after treatment

group	case	ET (ng/L)		CGRP (ng/L)	
		before	after	before	after
donepezil	65	67.41±7.45	55.71±6.96*	33.52±6.89	40.15±5.29*
galantamine	63	66.96±8.52	57.01±7.77*	32.89±7.15	39.63±4.96*
t value		0.318	0.998	0.508	0.573
P value		0.751	0.320	0.613	0.568

Note: Compared with before treatment, *P<0.05

3.4 Expression of neurotrophic molecules in both groups before and after treatment

CER, BDNF and miR-132 levels in both groups increased after treatment (P<0.05), and the levels of BDNF and miR-132 in donepezil group were significantly higher than those in galantamine group (P<0.05).

Table.5. Expression of neurotrophic molecules in the two groups before and after treatment ($\bar{x} \pm s$)

group	case	CER (mg/L)		BDNF (pg/ml)		miR-132	
		before	after	before	after	before	after
donepezil	65	0.17±0.05	0.21±0.05*	0.24±0.03	0.27±0.07*	0.55±0.10	0.70±0.15*
galantamine	63	0.17±0.04	0.20±0.04*	0.23±0.04	0.24±0.05	0.53±0.08	0.62±0.09*
t value		0.249	1.247	1.603	2.782	1.247	3.645
P value		0.803	0.205	0.111	0.006	0.215	0.001

Note: Compared with before treatment, $^{*}P<0.05$

3.5 Safety analysis

No serious adverse reactions were found in both groups, and the main adverse reactions were nausea and vomiting, abdominal pain, dizziness and fatigue, and abnormal liver function. Nausea and vomiting, abdominal pain and dizziness and fatigue were mild to moderate and transient, which were relieved after drug withdrawal or symptomatic treatment. 1 case in the donepezil group developed drug acute liver injury after treatment and recovered to normal after treatment. The overall incidence of adverse reactions in the donepezil group and the galantamine group was 16.92% and 15.87%, and there was no statistically significant difference between the two groups (P>0.05). See Table 6 for details.

Table.6. Analysis of the occurrence of adverse reactions in the two groups

group	case	Nausea vomiting	abdominal pain	dizziness fatigue	abnormal liver function	total
donepezil	65	3 (4.62)	2 (3.08)	5 (7.69)	1 (1.54)	11 (16.92)
galantamine	63	3 (4.76)	3 (4.75)	4 (6.35)	0 (0.00)	10 (15.87)
$\chi 2$ value		0.144	0.001	0.002	0.001	0.026
P value		0.705	0.972	0.961	0.987	0.873

4. Discussion

Cholinesterase inhibitors are the first drugs approved for the treatment of AD, and studies have shown that such drugs can delay the disability of AD patients, and may also reverse their physiological and pathological progress, so as to achieve the treatment goal. However, some patients have poor treatment results, which may be related to drug tolerance of cholinesterase inhibitors after long-term treatment. This study involved donepezil and galantamine are cholinesterase inhibitors. The two-drug treatment effect is observed. MMSE in the two groups after treatment increased and ADAS-cog decreased. No significant differences between the two group, suggesting the two drugs can improve cognitive function in patients with AD to a certain extent. The effects on the improvement of the cognitive function of the two drugs were similar. The evaluation of the daily living ability of the two groups also showed that the daily living ability of the two groups was similar, which was consistent with the trend of the cognitive function results. Previous studies have explored the effects of the two drugs in the treatment of AD, but there are few studies on the direct comparison of the two drugs, and the results are different: Zhu Li and others confirmed that donepezil and galantamine alone had similar effects on the improvement of cognitive function in AD patients. However, the study carried out by Wang Lihua and others confirmed that the increase of MMSE score after donepezil treatment was faster than galantamine; Other foreign studies have confirmed that donepezil's improvement effect on the cognitive function of AD patients is worse than galantamine. This study is similar to the results of Zhu Li's study, and the differences between the analysis and some studies may be caused by the difference in the degree of disease of the subjects, the dosage of drugs, and the evaluation methods of cognitive function.

Currently, studies on the efficacy of cholinesterase inhibitors are mostly focused on cognitive function evaluation, while there are few reports on serum markers. To explore the therapeutic effect

of drugs from serum markers is helpful to clarify the therapeutic effect more sensitive, and provide data support for the study of therapeutic mechanism. The mechanism of AD is complex, and it is currently believed that microvascular injury, oxidative stress, Tau protein hyperphosphorylation and Aβ protein deposition are closely related to neuron injury. In this study, ET, CGRP, CER, BDNF, miR-132 and other indicators were selected for evaluation. ET is an effector molecule with strong vasoconstriction and can be produced by endothelial cells, glial cells and other cells; CGRP is the strongest vasomotor substance, mainly released by peptide energy nerve fibers, which can regulate vascular elasticity and maintain local vasomotor function in the body. Previous studies have shown that vascular endothelial damage caused by imbalance of ET and CGRP levels is involved in the occurrence of various cognitive diseases. The results of this study showed that ET decreased and CGRP increased in the two groups after treatment, but there was no significant difference between the two groups, suggesting that the two drugs had similar effects on vasoactive factors. CER is a class of proteins with antioxidant effect, which can affect neuron injury and repair by affecting neurotransmitter synthesis. BDNF is a neurotrophic molecule that helps damaged nerves recover; miR-132 regulates the expression of BDNF and has also been shown to mediate Aβ-mediated nerve injury. The results of this study showed that the expressions of CER, BDNF and miR-132 in the two groups significantly increased after treatment, suggesting that the two drugs had certain neurotrophic and regulatory effects, which were similar to the results reported by Liu Dongmei and others. In comparison between the two groups, the levels of BDNF and miR-132 after donepezil treatment were slightly higher than those of galantamine, suggesting that donepezil may have a better effect on nerve repair. This result is different from the cognitive function evaluation mentioned above. It is speculated that these differences may not be manifested in the macro level, or there may be other mechanisms involved in the impairment of cognitive function.

Adverse reactions of cholinesterase inhibitors are mainly related to cholinergic activity. Common clinical adverse reactions include digestive system and central nervous system. In this study, adverse reactions include nausea, vomiting, dizziness, etc., which is similar to studies at home and abroad; In this study, the adverse reactions in the two groups were mild to moderate, and most of them were transient. The safety of the two groups was similar. However, recent studies have reported that the use of acetylcholinesterase inhibitors also has an impact on cardiac repolarization and other functions. For example, studies have shown that donepezil can prolong cardiac repolarization, but galantamine has no significant change. In this study, the overall evaluation of organ function was only carried out, and the duration of medication was short, so no obvious serious adverse reactions were observed. Attention should be paid to the assessment of organ function in the case of prolonged use.

5. Conclusion

Donepezil and galantamine have good efficacy and safety in the treatment of mild to moderate AD, but donepezil may have some advantages in improving neurotrophic molecules, which needs to be confirmed by larger samples and longer follow-up studies.

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