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Original Article

Tanshinones encapsulated PLGA nanoparticles: preparation and the effect on cognitive improvement with the combination of borneol

Wang Di[1], Li Wei[2], Wu Yihan[1], Zhan Gu[1], Zhang Chen[1], Gao Huile[3], Zou Liang[4], Zhang Jinming[1]

[1] *State Key Laboratory of Southwestern Chinese Medicine Resources, School of Pharmacy, Chengdu University of Traditional Chinese Medicine, China*

[2] *School of Basic Medicine, Chengdu University, China*

[3] *West China School of Pharmacy, Sichuan University, China*

[4] *Key Laboratory of Coarse Cereal Processing, Ministry of Agriculture and Rural Affairs, Chengdu University, China*

Abstract: The cognitive impairment belongs to the most comment symptom caused by several central nervous system (CNS) diseases, leading to the lowered living quality. However, these commonly therapeutic agents scarcely exhibit the satisfactory clinical outcome, due to the unclear pathogenesis as well as the delivery challenges posed by blood-brain barrier. The purpose in this study is to develop a therapeutic approach with the combination of tanshinones co-loaded nanoparticles (NPs) and borneol, to improve the cognitive impairment. Primarily, tanshinone IIA (TanIIA) and cryptotanshinone (CTan), which are the main tanshinones derived from traditional Chinese herbal *Salvia miltiorrhiza* Bunge, were loaded into PLAG copolymer (TC@PLGA/NPs) by one-step solvent evaporation method. TC@PLGA/NPs exhibited the encapsulation efficiency of TanIIA and CTan were 85.31±1.28% and 86.42±2.07%, respectively. LE% of TanIIA and CTan were 1.24±0.09% and 1.53±0.15%, respectively. Meanwhile, it had the average particle size of 194.2±3.5 nm and good storage stability. The DSC results indicated that the tanshinones were well encapsulated in PLGA polymer, instead of the physical mixture. The Morris water maze (MWM) behavioral experiment demonstrated that TC@PLGA/NPs with the combination of borneol (BNL+TC@PLGA/NPs) could dramatically improve the spatial learning and memory capability of AD model rats, compared with other groups. Specifically, the administration of BNL+TC@PLGA/NPs can significantly shorten the escape latency and swimming distance of scopolamine-induced dementia rats, meanwhile it can also reduce the expression of MDA and AchE in hippocampus. In conclusion, combined the advantage of borneol to enhance drug delivery across BBB and the tanshinones co-loaded NPs, the learning ability and cognitive function could be significantly improved. It would provide a useful strategy for CNS diseases treatment.

Keywords: tanshinone IIA; cryptotanshinone; borneol; cognitive impairment; PLGA

Corresponding authors: Zou Liang: zouliangcdu@126.com;
Zhang Jinming: cdutcmzjm@126.com

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Introduction

Alzheimer's disease (AD) is one of the most serious aging-related neurodegenerative brain disorders, manifested primarily by progressive cognitive impairment, memory impairment, and affective disorders.[1] It is currently occurring in approximately 40 million people globally.[2,3] The main pathology of AD is predominantly characterized by extracellular plaque formation of β-amyloid (Aβ), neurogenic fiber tangles (NFT) and neuronal cell death, appearing in the frontal and temporal lobes of the cerebral cortex, hippocampus, and basal forebrain. Despite the unclear etiology of AD, it is generally believed that the formation of Ab plaques leads to NFT, causing neuronal cell death and cognitive impairment. Memory disorders are associated with cholinergic neuron dysfunction in AD. In addition, many studies have shown that inflammation, cholinergic nerve damage and oxidative stress also contributes to the incidence of AD.[4-6] At present, due to its complex pathogenesis, there is a lack of effective treatment methods for AD.[7] A variety of treatment medications are currently being developed to treat AD, such as cholinergic inhibitors, N-methyl-D-aspartate receptor antagonist, and some AD treatment that promote blood flow in the cerebral blood vessels. However, these drugs have hepatotoxicity and gastrointestinal adverse effects, combined with failure to stop progressive neuroinflammatory dystrophy, costing a high price of treatment and limiting its clinical efficacy. Therefore, it is urgent to develop an alternative agent's multi-target drug to prevent and treat AD.

The effectiveness of TCM in preventing and controlling neurological diseases is gradually goes into people's visual field. [8,9] The root of *Salvia miltiorrhiza* (Lamiaceae), known as "Danshen" in Chinese, has been widely applied in the treatment of cardiovascular and cerebrovascular diseases. [10,11] Clinical studies have found that Danshen has anti-inflammatory, improves microcirculation and protects the central nervous system. The main components of Danshen can be divided into two groups, including water-soluble polyphenolic compounds and lipophilic chemicals, with both groups contribute to the biological activities of Danshen. Studies have shown particularly lipophilic chemicals, such as tanshinone IIA (TanIIA) and cryptotanshinone (CTan) have significant multiple neuroprotective potentials related to AD.[12-14] TanIIA can inhibit oxidative stress response to improve learning and memory function of AD rats. In addition, TanIIA has anti-Aβ, anti-inflammation, and induces neurogenesis of neural progenitor cells/stem cells in vitro and in vivo. Meanwhile, CTan can reduce the deposition of Tau protein in the brain, and enhancement of cholinergic signaling to prevent AD. These results indicate that both components show great potential in the treatment of AD.[15-17] In order to make TanIIA and CTan pass through the blood-brain barrier (BBB) and produce better results, nano-scaled carriers have been considered as the most promising tools with various advantages on increasing stability, bioavailability and targetability for the treatment of brain diseases.[18,19] Among diverse nano-vehicles, polymer materials are ideal artificial carriers with superior biodegradability and targetability.[20] Previous studies have confirmed the potential advantages of TanIIA/CTan encapsulated in poly (lactide-co-glycolide (PLGA)) nanoparticles (TC@PLGA/NPs) for improving the oral bioavailability of insoluble drugs.[21] Although nanopreparations improve accumulation capacity and bioavailability of the drugs at the brain compared to free drugs. However, complex, and functional BBB

can protect brain tissue while limiting the substances transport into the brain lead to poor bioavailability. Borneol (BNL), possessing the ability to the permeability of BBB, is widely used in the treatment of cardiovascular and cerebrovascular diseases. Previous studies have reported that it can be used in combination with certain drugs, such as ligustrazine, geranium and asparagine, to increase the permeation and accumulation in the brain, achieving the effect of improving the bioavailability of the drug. In addition, in our previous work, we found that borneol has an obvious permeation effect on blood-brain barrier and can improve the bioavailability of gastrointestinal drugs. [22] Consequently, borneol were employed by us to assist in enhancing the ability of PLGA nanoparticles (NPs) to penetrate the BBB. The new drug delivery system (BNL+TC@PLGA/NPs) was prepared by combining the characteristics of polymer nanoparticles and borneol. Based on the optimization and characterization of TC@PLGA/NPs, the combination of borneol were employed to evaluate the effects on spatial memory in MWM rat test, followed by the determination of malonaldehyde (MDA) content and acetylcholinesterase (AchE) expression in hippocampus.

1. Materials and methods

1.1 Materials

Tanshinone IIA (TanIIA, purity≥98%), Cryptotanshinone (CTan, purity≥98%) and natural Borneol (BNL, purity≥98%) were purchased from Melonephama Co., Ltd. (Dalian, China). PLGA (d,l-lactide-co-glycolide, MW=15000, lactide: glycolide (75:25)) was obtained from Dai-Gang Pharma Co., Ltd. (Shandong, China). Scopolamine hydrobromide was provided by Karamay Reagent Co., Ltd. (Shanghai, China). Polyvinyl alcohol (PVA, alcoholysis degree: 88%, MW=22000) was from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade.

Ten-month-old male SD mice weighing 280-320 g were purchased from Dashuo Laboratory Animal Reproduction Center (Chengdu, China). All mice were kept under pathogen-free environment and allowed free access to the diet and water. All methods were carried out in accordance with relevant guidelines and regulations and procedures involving mice were approved by the animal care center of Chengdu University.

1.2 Preparation and characterization of TanIIA/CTan-loaded PLGA Nanoparticles (TC@PLGA/NPs)

1.2.1 Drug loading in PLGA/NPs

TanIIA and CTan (TC) were jointly encapsulated in PLGA NPs by solvent evaporation method with assistance of ultra-sound as reported studies.[23] Some vital preparation parameters were optimized by orthogonal design tests with encapsulation efficiency (EE%), loading efficiency (LE%) and nanoparticle size as comprehensive evaluation index. More specifically, the model drug (TC,TanIIA:CTan=1:1) and PLGA were dissolved by ultra-sound to blend well with dichloromethane in different mass ratios (20:1, 25:1, 30:1). Then, the oil phase dropped in a certain ratio of PVA solution consisting of aqueous phase (1%, 1.5%, 2%). The mixed solution was dissolved by ultrasonic cell breaker (UCB, Shanghai Qiqian Electronic Technology Co., LTD., Shanghai, China) with a certain time (4, 6, 8 min) and different ultrasonic power (500, 600, 700 W). After ultrasonic treatment, the nano suspension was placed on magnetic stirrer and stirred at room temperature for 2 hours to remove the organic solvent from the nano-particle solution. At last, the total TC@PLGA/NPs was obtained by centrifugation(Centrifuge, Changsha Yingtai Instrument Co., LTD, Changsha, China) at

13000 rpm for 30 minutes and freeze drying (Freeze dryer, VIRTIS Instrument Co., LTD, USA) technique.

1.2.2 Characterization of TC@PLGA/NPs

TC@PLGA/NPs were diluted properly with distilled water at the polymer concentration of 1 mg/ml, and the particle size, polydispersity index (PDI) and zeta potential were determined by a nano particle size analyzer (NSA, Malvern Instruments Ltd., Worcestershire, UK). The TC@PLGA/NPs were dripped on copper net for 10 min and dried with filter paper. Then it was stained with 2.0% phosphor tungstic acid solution for 5min to analyze the morphology of prepared TC@PLGA/NPs by a Tecnai G20 transmission electron microscope (TEM, FEI, Hillsboro, OR). The storage stability of TC@PLGA/NPs was evaluated by the particle size change, PDI and encapsulation efficiency EE% at 4 ℃ during 15 days. In order to measure the EE% and loading efficiency LE% of drugs, the TC@PLGA/NPs were dissolved in methanol to disrupt the polymeric shells before analysis. Then, the concentration of TanIIA and CTan were determined by Waters UPLC (UPLC, Waters Instruments Ltd., USA) equipped with a reverse phase C18 Column (2.1 mm×50 mm, 1.7 μm) at a maximum absorbance of 270 nm with methanol/water (75/25, v/v) as the mobile phase at a flow rate 0.3 ml/min. The EE% and LE% were calculated using the following equations, respectively:

$$EE(\%)= \frac{\text{amount of drug loaded}}{\text{amount of drug loaded}} \text{ x100\%}$$

$$LE(\%)= \frac{\text{amount of drug loaded}}{\text{amount of drug loaded+polymer}} \text{x100\%}$$

The samples to be analyzed by differential scanning calorimetry (DSC) (DSC, Shanghai Hesheng Instrument Technology Co., LTD, Shanghai, China) with the scanning range of 0~250 ℃ at a heating rate of 10 ℃ /min.

1.3 Pharmacodynamics test of animals in vivo

1.3.1 Animals

A total of 64 healthy male Sprague-Dawley rats, aged ten months and weighted 300±20 g, were purchased from Dashuo Laboratory Animal Reproduction Center (Chengdu, China). All rats were adopted to the laboratory environment (23±0.5 ℃, relative humidity 55±3%) and allowed free access to the diet and water. Before treatment, animals were given a 3-day acclimation period to the laboratory condition. All in vivo experiments were conducted in accordance with the Guidelines for Nursing and Use of Laboratory Animals of National Institute of Health of Chengdu University.[24]

1.3.2 Dose and grouping

All the 64 rats were randomly divided into 8 groups(8 rats in each group), as follows: (a) control group; (b) model group; (c) donepezil group (1 $mg{\cdot}kg^{-1}$); (d) blank NPs group; (e) BNL group (20 $mg{\cdot}kg^{-1}$); (f) free drug of TC group (TanIIA : 4 $mg{\cdot}kg^{-1}$; CTan : 5 $mg{\cdot}kg^{-1}$); (g) TC@PLGA/NPs group (TanIIA : 4 $mg{\cdot}kg^{-1}$; CTan : 5 $mg{\cdot}kg^{-1}$); (h) BNL+TC@PLGA/NPs group (BNL : 20 $mg{\cdot}kg^{-1}$; TanIIA : 4 $mg{\cdot}kg^{-1}$; CTan : 5 $mg{\cdot}kg^{-1}$). BNL and free drug of TC were dissolved in CMC-Na. Each group was administered once a day by gavage for 28 days. Except the control group, all rats in each group were injected with 3 $mg{\cdot}kg^{-1}$ scopolamine solution from the 22nd day after intragastric administration for 1 hour, so as to make Alzheimer's disease model. Meanwhile, from the 24th day, each group was tested by Morris water maze (MWM) 20 minutes after scopolamine injection for 5 days.

1.3.3 Behavioral test of rats

All rats in each group were tested for behavior by MWM consisted of a circular pool filled with water (22±2 ℃) to a depth of 50 cm and divided into four equivalent quadrants, including north-east (NE), north-west (NW),

south-east (SE), and south-west (SW), a platform located 1 cm below the water surface in the center of the target quadrant (NW). This experiment lasted for 5 days, including 4-day positioning navigation experiment and space exploration experiment for a day.

Escape latency and total traveling distances, respectively, were used as indicators to evaluate the learning ability of experimental rats in the positioning navigation experiment. Escape latency, as one of the indexes, refers to the time taken by rats to enter water from different quadrants to find the central platform of the second quadrant in 90 seconds. If a rat fails to reach the platform within 90 seconds, the experimenter will guide it to the platform and stay on the platform for 15 seconds, and its escape latency was 90 seconds.

In space exploration experiments, the hidden platform was removed, and the rat was placed on the NW quadrant and allowed to swim for 90 seconds. The 4 indicators we selected, time in target quadrant, residence time in platform, travel distances in quadrant and number of platform crossing, to evaluate the memory function of rats systematically.

1.3.4 Determination of MDA contents in hippocampus

All rats were sacrificed by euthanasia. The hippocampus tissues were collected and cleaned with cold PBS. 0.9% sodium chloride solution was rapidly added to the right side of hippocampus to obtain the 10% tissue homogenate through tissue homogenate on ice water bath. MDA content in hippocampus homogenate suspension was determined. And then, the left part of the hippocampus was put into 4% paraformaldehyde for 24 hours, and a series of operations such as gradient alcohol dehydration, xylene removal and paaffin embedding were performed. Paraffin section from the similar section from each rat was used for hematoxylin and eosin (H&E) staining. The histo-morphological chanes of the hippocampus were observed with micrscope at x100 and x400 magnification.

1.3.5 Expression of AchE in hippocampal tissue

The expression of AchE in hippocampus was determined by immunohistochemical method using the specific binding between antigen and antibody. First, the dewaxed slices were stained with methanol and hydrogen peroxide at 25℃ for 10 minutes. The slices were washed in PBS for 15 minutes, and then immersed in 0.01 m citrate buffer. Then, heat up to boiling and cool it. Then 10% goat serum blocking solution, first antibody and biotinylated secondantibody were added in turn. Similarly, the next series of operations have been described before, such as gradient alcohol dehydration, removal in xylene and neutral glue sealing sheet. Finally, the average optical density (Image Pro Plus, IPP) of the collected images was calculated.

1.4 Statistical analysis

The SPSS 19.0 Software (IBM Corp., Armonk, NY, USA) and OriginPro 9.1 Software (OriginLab Corp., Northampton, MA, USA) were applied to data analysis. Besides, all results were expressed as the mean±standard deviation. Comparisons between two groups were conducted by independent t-test; and comparisons among multiple groups were performed using the one-way ANOVA test. $p<0.05$ considered statistically significant.

2. Results

2.1 Preparation and characterization of TC@PLGA/NPs

TC@PLGA/NPs were prepared by emulsion solvent evaporation method. The formula was optimized by L18 (35) orthogonal design and calculated by UPLC (Figure 1) with three levels of factors. Particle size, EE% and LE% were as the comprehensive evaluation indexes. The results and variance analysis are shown in Tables 1 and 2. The data analyzed by comprehensive weighted scoring method. The maximum particle size, encapsulation efficiency and drug loading were determined to be 100, and the weight coefficients were 0.5, 0.3 and 0.2 respectively. According to results, the contribution of the ratio of PLGA to drugs and volume ratio of oil phase to aqueous phase are significant. The optimum preparation process of TC@PLGA/NPs was deduced as NO.3: PLGA-drugs ratio of 20:1, PVA concentration of 1.5%, oil phase-aqueous phase ratio of 1:15, ultrasonic time of 8 min and ultrasonic power of 700 W.

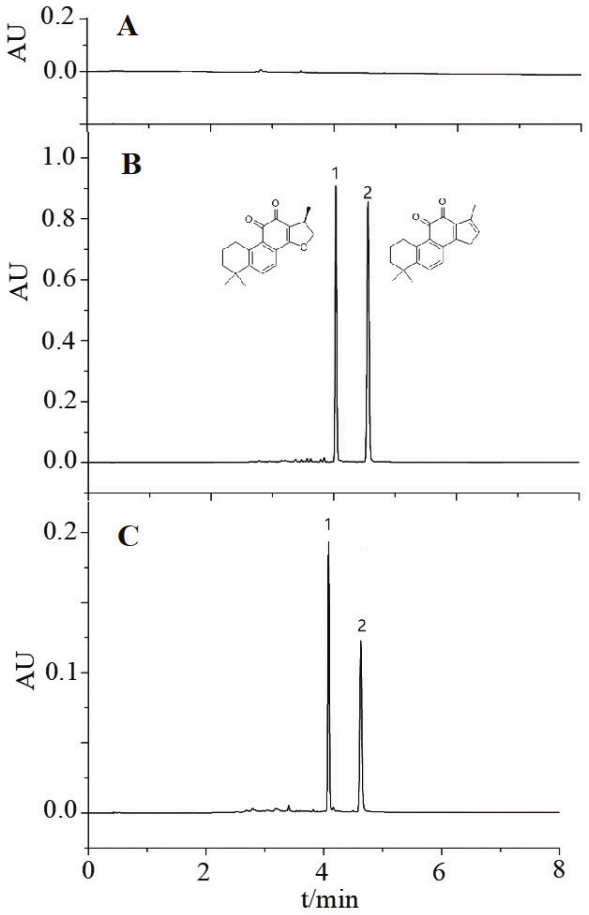

Figure 1 UPLC spectrum of blank NPs(A), mixed reference substances of TC(B) and TC@PLGA/NPs(C).

Note: 1 : CTan ; 2 : TanIIA

Table 1 Orthogonal test of L18(35) of TC@PLGA/NPs preparation optimization

NO	A	B	C	D	E	Diameter (nm)	EE (TanIIA%)	EE (CTan%)	LE (TanIIA)%	LE (CTan%)	Overall score
1	1	1	1	1	1	236.0±1.7	74.84%	88.48%	0.67%	1.28%	45.91
2	1	2	2	2	2	250.3±3.6	81.79%	81.53%	1.40%	1.65%	50.59
3	1	3	3	3	3	190.9±1.7	83.46%	84.02%	1.26%	1.57%	60.35
4	2	1	1	2	2	225.3±3.2	80.59%	82.21%	0.48%	1.02%	45.04
5	2	2	2	2	3	206.1±2.7	71.91%	75.42%	0.72%	1.23%	48.77
6	2	3	3	1	1	208.2±2.9	80.21%	79.37%	1.04%	1.29%	52.98
7	3	1	2	1	3	225.6±2.7	74.06%	73.64%	0.42%	0.79%	40.93
8	3	2	3	2	1	236.3±3.5	86.76%	88.34%	0.79%	1.21%	48.28

Table 1 Orthogonal test of L18(35) of TC@PLGA/NPs preparation optimization (cont.)

NO	A	B	C	D	E	Diameter (nm)	EE (TanIIA%)	EE (CTan%)	LE (TanIIA)%	LE (CTan%)	Overall score
9	3	3	1	3	2	252.4±4.0	77.74%	81.52%	0.54%	1.05%	40.25
10	1	1	3	3	2	210.8±2.7	83.83%	89.25%	1.32%	1.90%	59.88
11	1	2	1	1	3	242.4±2.1	84.51%	88.44%	0.60%	1.50%	47.02
12	1	3	2	2	1	256.3±5.5	77.94%	83.24%	0.80%	1.45%	43.83
13	2	1	2	3	1	254.1±2.1	83.56%	87.33%	0.64%	1.23%	43.48
14	2	2	3	1	2	208.5±3.3	80.93%	88.75%	1.08%	1.56%	56.23
15	2	3	1	2	3	230.2±6.5	81.83%	83.69%	0.45%	1.22%	45.45
16	3	1	3	2	3	242.5±0.6	91.47%	82.91%	0.45%	1.21%	44.67
17	3	2	1	3	1	280.3±3.4	91.49%	95.44%	0.54%	0.75%	38.18
18	3	3	2	1	2	282.4±6.0	74.83%	76.27%	0.51%	1.01%	33.21

Table 2 The result of variance analyses

Factor	SST	F	p
A	347.305	2.923	p<0.05
B	14.87	0.125	p>0.05
C	414.348	3.487	p<0.05
D	21.491	0.181	p>0.05
E	20.685	0.174	p>0.05

Note : Freedom(f)=2

A: PLGA-drugs ratio, B: PVA concentration, C: oil phase-aqueous phase ratio, D: ultrasonic time, E: ultrasonic power.

Optimum preparation process was repeated three times under this condition. We obtained the TC@PLGA/NPs with a homogeneous translucent colloid appearance and a good storage stability, which were characterized by a mean particle size of 194.2±3.5 nm with a narrow distribution (PDI=0.189), and the zeta potential of -15.5 mV. The TEM images (Figure 2B) showed that TC@PLGA/NPs exhibited the uniform particle size, smooth surface and spherical shape (Figure 2A and Figure 2B). In addition, the EE% of TanIIA and CTan were (85.31±1.28)%, (86.42±2.07)%, the LE% of TanIIA and C Tan were (1.24±0.09), (1.53±0.15)%, respectively. Meanwhile, the storage stability was verified by the change of particle size, PDI, and EE% once again during 15 days storage. As shown in Figure 2C and Figure 2D, there was no obvious change from the appearance after 15 days, EE% and particle size are basically consistent on the whole. Only the EE% of TanIIA showed a small decline during the period from day 5 to day 15.

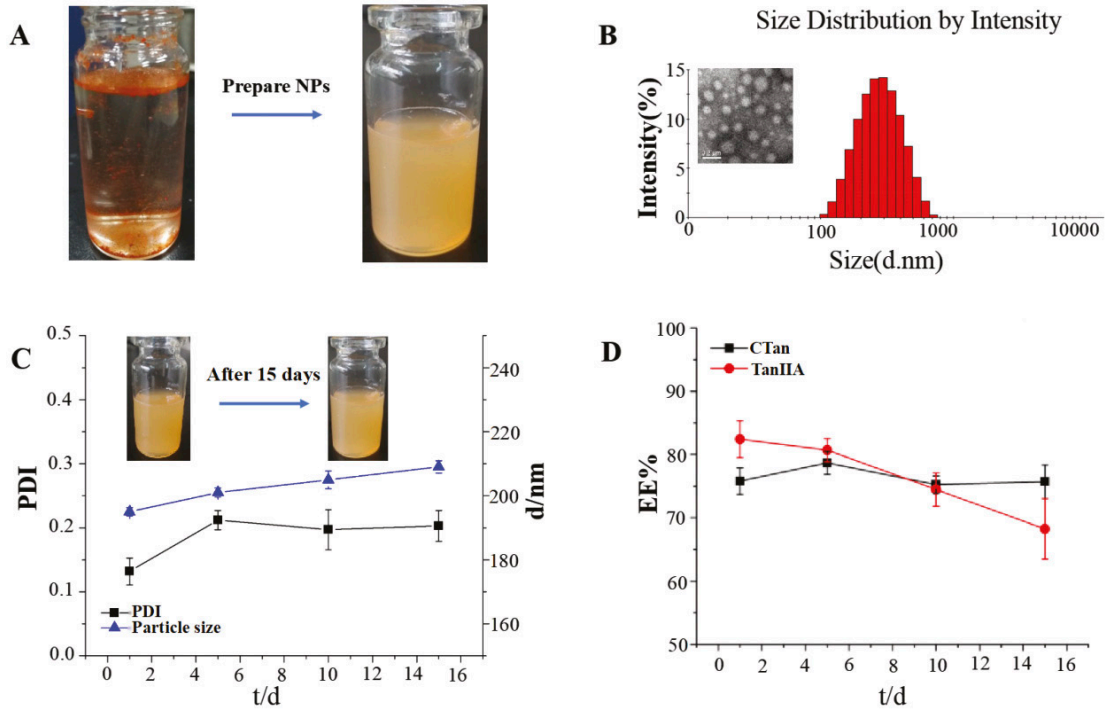

Figure 2 Appearance (A), size distribution and transmission electron microscopic images (B) of NPs, the variable trend graph of particlesize (C) and EE% (D) of TC@PLGA/NPs in 15 days of TC@PLGA/NPs.

The chemical interactions after encapsulation of TC into PLGA NPs were investigated by DSC. As shown in Figure 3, apparently, there was a sharp endothermic peak of PLGA at 140 degrees Celsius. In addition, the free drug TC appeared another endothermic peak at around 180 degrees Celsius. Obviously, the spectrum of its physical mixture was the superposition of two curves, and the glass-transition tempeature was close to that of the TC and PLGA. However, great changes had taken place the TC@PLGA/NPs in characteristic peak compared with the spectrum of its physical mixture, which had no characteristic peak of free TC. The glass transition temperature is also greatly reduced. The results showed that the original crystal form of TC loaded in PLGA nanoparticles changed.

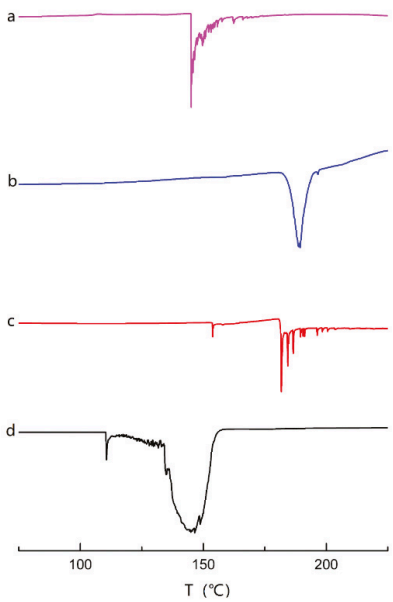

Figure 3. DSC curve of different samples.

Note: a. Blank NPs; b. Free TC;

c. Physical mixture; d.TC@PLGA/NPs

2.2 Behavioral tests

As seen in the figure 4A, the swimming trajectory of the saline control group was mostly linear. However, the swimming trajectories in the model group mostly showed marginal type. Except blank NPs, other groups tend to be directional. The results showed that rats in normal saline control group can locate quickly and accurately, and landed safely. During the training period, rats in the model group spent more time looking for the platform than those in other groups in the positioning navigation experiment (Figure 4B). There was a significant difference ($p<0.05$) between the model group and the TC@PLGA/NPs group. In addition, as shown in Figure 4C, there is a significant difference between model group and TC@PLGA/NPs group in the total distance index.

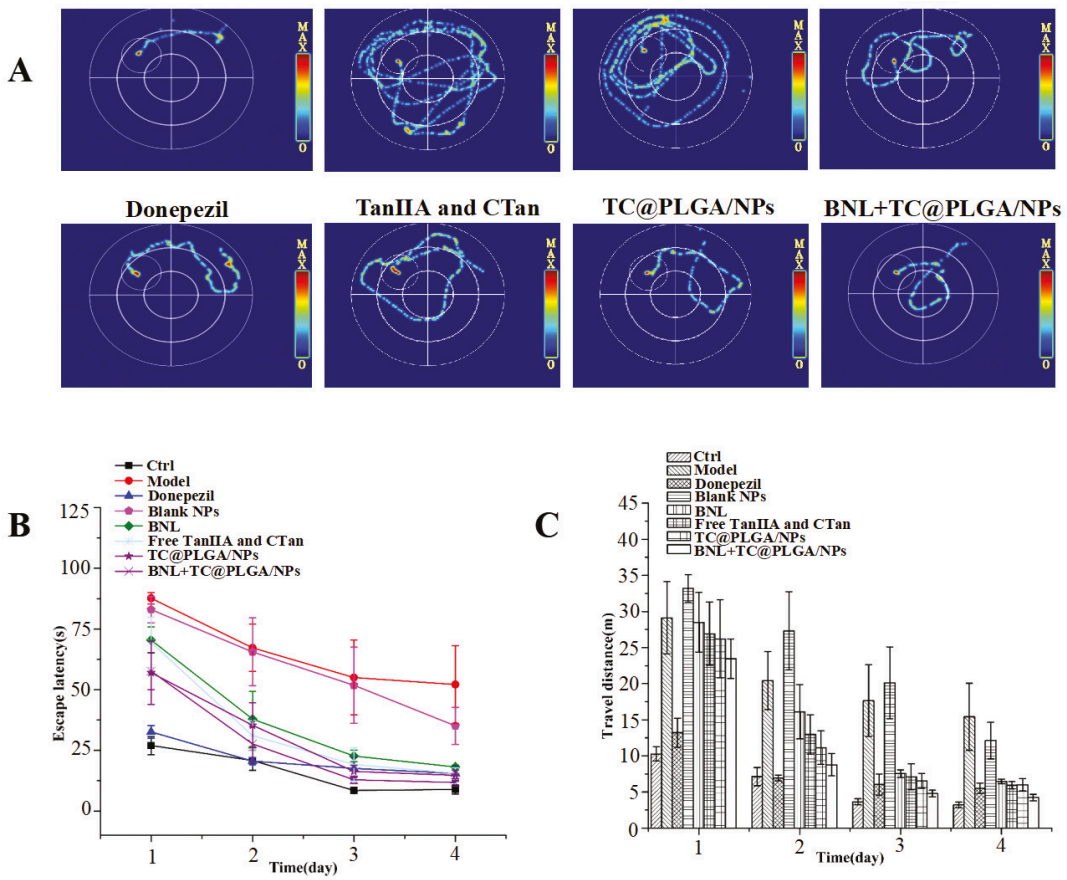

Figure 4 Swimming trace (A), escape latency (B) and swimming distances (C) of rats in the positioning navigation stage.

Through space exploration experiment, the target quadrant time, platform stay time, quadrant travel distance and platform crossing times were tested, and the learning and memory ability of rats were comprehensively evaluated, as shown in Table 3 and Figure 5. The results showed that compared with the saline control group, the rats in saline control group were more concentrated in the target quadrant within 90 seconds, and the rats in model group was more disordered and the target was unclear. Compared to the model group, all the treatment groups improved in four different evaluation indexes (except the blank control group). Especially, the BNL+TC@PLGA/NPs group improved memory function of dementia rats more significantly.

Table 3 Four different evaluating indicators in space exploration experiment

Group	residence time in platform(S)	number of platform crossing	time in target quadrant(S)	travel distances in quadrant (m)
Saline ctrl	9.96±5.68#	10.80±4.49#	34.14±3.36	8.32±2.02
Model	1.88±1.23**	3.60±3.21*	25.32±4.11*	7.15±0.28
Donepezil	10.08±3.04#	10.40±3.36#	29.26±4.27	7.62±1.09
Blank NPs	8.13±6.29	8.67±6.03	24.73±6.49**	7.20±1.25
BNL	8.52±6.14	11.00±5.57##	28.10±7.45	7.83±1.04
Free TC	8.5±5.35	10.60±6.19##	27.24±5.81	7.37±0.27
TC@PLGA/NPs	11.30±6.93#	9.60±5.86	29.28±7.32	7.80±0.66
BN+TC@LGA/NPs	11.94±3.62#	14.40±2.88##	36±7.28##	8.35±0.79

Note: Compared with the saline ctrl group: * p<0.05, ** p<0.01; compared with the model group, #p < 0.05, ##p<0.01

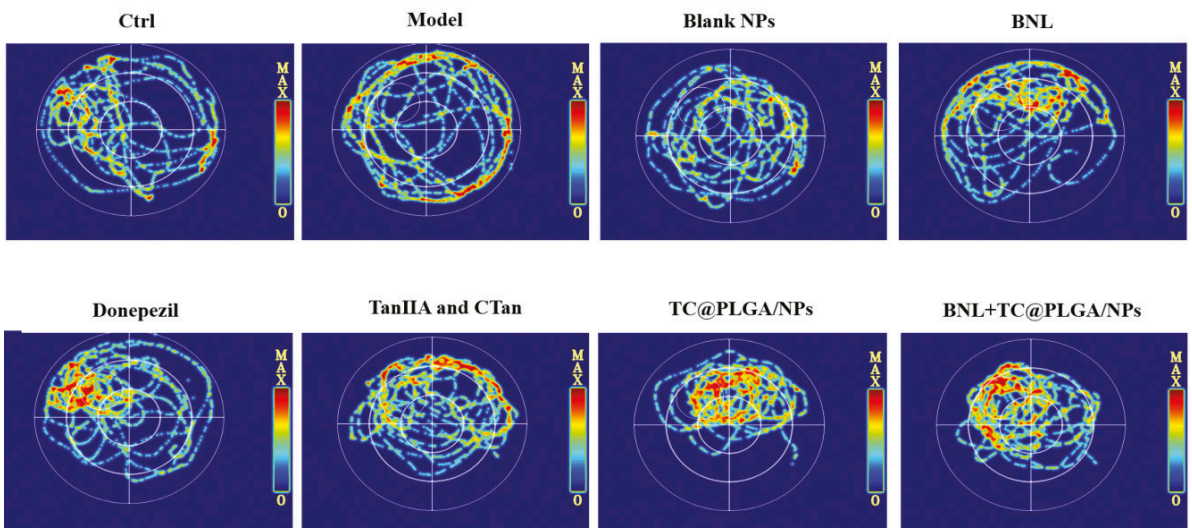

Figure 5 The swimming trace of rats in the space exploration stage

2.3 Determination of MDA contents in hippocampus

As shown in Table 4, compared with the model group, the content of MDA in hippocampus of other groups decreased compared with the model group. Especially, the BNL+TC@PLGA/NPs group decreased significantly (p<0.05). This indicates that BNL combination would significantly reduce the content of MDA in hippocampus.

Table 4 Content of MDA in hippocampus of rats in each group

Group	MDA contents (nmol/mgprot)
Saline ctrl	1.664±0.062
Model	1.820±0.086*
Donepezil	1.611±0.300
Blank NPs	1.682±0.205
BNL	1.667±0.077
Free TC	1.645±0.596
TC@PLGA/NPs	1.631±0.271
BNL+TC@PLGA/NPs	1.599±0.007#

Note: Compared with the saline ctrl group: * p<0.05; compared with the model group, #p<0.05.

2.4 H&E staining in hippocampus

As shown in Figure 6, all the pyramidal cells in the hippocampus of normal saline control group were arranged closely and orderly, and there was no cell necrosis and rapid changes of cell numbers. In addition, the hippocampal formation is basically normal, the stroma is dense, without edema. Compared with normal saline control group, the rats in model group had different pathological changes. It mainly includes constriction and necrosis in pyramidal cells.Compared with the model group, each administration group improved the morphology of the hippocampal cells in scopolamine model rats in varying degrees. Surprisingly, the BNL+TC@PLGA/NPs group showed the most significant improvement on the pathological changes of hippocampal tissue cells in scopolamine-induced dementia rats, and its therapeutic effect even exceeded that of Donepezil.

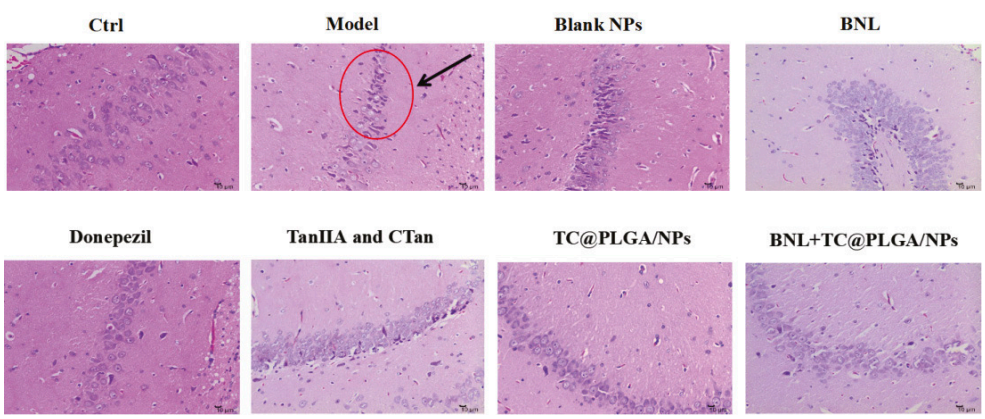

Figure 6 H&E staining in hippocampus of rats in each group

2.5 Expression of Ache in hippocampal tissue

It can be seen from Figure 7A that compared with the normal saline control group, the positive cells in the hippocampus of the model group showed dark brown, indicating that the expression of AchE was significantly enhanced. Compared with the model group, the expression of AchE in different administration groups decreased to some extent. The quantitative analysis in Figure 7B shows that compared with normal rats, the integrated optical density (IOD) of AchE in hippocampus of dementia rats increased significantly. Treatment with BNL+TC@PLGA/NPs resulted in significant decrease in AchE activity ($p<0.05$). Remarkably, the correlation analysis showed that there was a strong negative correlation between IOD and retention time of target quadrant, which indicated that decreasing the expression of AchE in hippocampus could improve memory and cognitive function of dementia rats (Figure 7C).

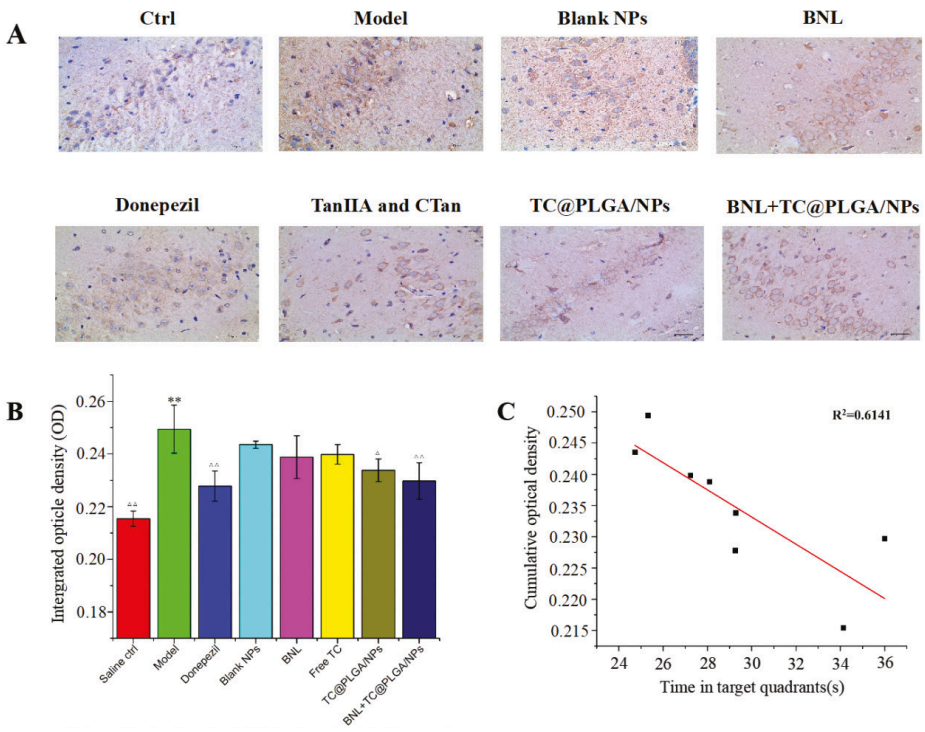

Figure 7 The expression of AchE in hippocampus(A) and the cumulative optical density(B) and correlation analysis between OD and the time in target quadrant(C).

Note: Compared with blank control group, *p<0.05 **p<0.01; Compared with model

3. Discussion

In the present study, we prepared TC@PLGA/NPs by combining nanotechnology with traditional Chinese medicine. The TC@PLGA/NPs have high encapsulation efficiency, moderate particle size and good stability. In addition, the results of differential scanning calorimetry fully indicate that drug crystal have changed in the nano-precipitation process. In the behavioral experiment, compare the free TC, TC@PLGA/NPs can significantly shorten the escape latency and swimming distance of scopolamine-induced dementia rats. In terms of platform residence time, TC@PLGA/NPs is 1.33 times higher than free TC. Meantime, TC@PLGA/NPs can also reduce the expression of AchE in hippocampus, so as to improve the learning ability and cognitive function.

The results of this study provide new strategies for the treatment of brain diseases.

Besides, a study has shown that borneol can enhance the bioavailability of several other drugs by opening the BBB and inhibiting P-glycoprotein (P-gp) efflux. Therefore, the combination of borneol, TanIIA and CTan loaded PLGA nanoparticles can further enhance the drug concentration in the brain. As shown in Figure 7, the combination of borneol and TanIIA and CTan loaded PLGA nanoparticles could lower the expression of AchE in hippocampus and improve the memory of rats significantly.

Author contributions

Wang Di and Li Wei contributed equally to this work. Wang Di prepared and characterized the PLGA nanoparticles. Li Wei and Gu Zhan conducted experiments on rat behavior. Zhang Chen and Wu Yihan worked for immunohistochemistry and pathology experiments. Zou Liang and Zhang Jinming gave the ideas and designed the whole research. All the authors were involved in the data analysis.

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References

1. Nezerwa M, Chen T, Fried-Stahl J, Joshi N, Terranova J, Wright R, et al. Universal design with mobile app development: bridging the gap for the forgotten populations. In: 2015 Long Island Systems, Applications and Technology Conference; 2015 May 1; Farmingdale, NY, USA. New York: IEEE; 2015. p. 1-6.
2. Klich-Rączka A, Piotrowicz K, Mossakowska M, Skalska A, Wizner B, Broczek K, et al. The assessment of cognitive impairment suspected of dementia in Polish elderly people: results of the population-based PolSenior Study. Exp Gerontol. 2014;57(9):233-42.
3. Sabir S, Roohi N. Association of electrophoretically resolved low molecular weight protein fractions with senile dementia in elderly people. Pak J Zool. 2011; 43(4):721-26.
4. Tian BJ, Wei WS. The contribution of vascular factors to the pathogenesis of alzheimer's disease. Chinese J Clinical Neurosciences. 2014;22(4):445-51. (in Chinese)
5. Wang Y, Liu ZB, Niu WM. Influence of “Xiusanzhen” electroacupuncture on spatial memory ability and APP, Aβ protein expression in hippocamus of mice with Alzheimer's disease. Shaanxi J Traditional Chinese Medicine. 2018;39(2):139-42. (in Chinese)
6. Li H, Liu JG, Yao MJ, Zhao WM. Effect of Huannaoyicong prescription to AD rat in learning and memory and hippocampus of Aβ-related protein expression with lipid metabolism. Chinese Journal of Basic Medicine in Traditional Chinese Medicine. 2009;15(5): 354-7. (in Chinese)
7. Lei X, Wang JH, Cheng XR, Zhou WX. Multiple-target agents in the therapy of Alzheimer's disease: research advances. J Int Pharm Res. 2016;43(2):205-15. (in Chinese)
8. Xiao XH, Niu M, Wang J. Precision medicine oriented drug R&D and quality control of traditional Chinese medicine. Modernization of Traditional Chinese Medicine and Materia Medica-World Science and Technology. 2017;19(6):900-5. (in Chinese)
9. Shen CY, Jiang JG, Yang L, Wang DW, Zhu W. Anti-aging active ingredients from herbs and

nutraceuticals used in traditional Chinese medicine: pharmacological mechanisms and implications for drug discovery. Br J Pharmacol. 2017;174(11): 1395-425.

10. Liu N, Guo L, Yang J. Role of syndrome elements to name Alzheimer's disease syndrome. Global Traditional Chinese Medicine. 2012;5(7): 517-20. (in Chinese)
11. Cheng Y, Feng J, Yao Q, Wu YH, Zhao FX. Discussion on the treatment of chronic arthritis by blood stasis. Guide of China Medicine. 2013;11(20):275-6. (in Chinese)
12. Qu Q, Zhang H, Zhao XP, Zhang GS. Professor Pei zhengxue's clinical experience on usage of *Salvia miltiorrhiza*. World Chinese Medicine. 2013;8(11):1326-8. (in Chinese)
13. Xu SX, Zhong AQ, Ma HN, Li D, Hu Y, Xu YZ, et al. Neuroprotective effect of salvianolic acid B against cerebral ischemic injury in rats via the CD40/NF-ÎºB pathway associated with suppression of platelets activation and neuroinflammation. Brain Res. 2017;1661: 37-48.
14. Jiang P, Li C, Xiang Z, Jiao B. Tanshinone IIA reduces the risk of Alzheimer's disease by inhibiting iNOS MMP-2 and NF-κBp65 transcription and translation in the temporal lobes of rat models of Alzheimer's disease. Mol Med Rep. 2014;10(2):689-94.
15. Jiang P, Sun XJ, Xiang ZH, Su XP, Li CB. The effect of tanshinone on the expression of iNOS MMP-2 in temporal of Alzheimer's disease model rats and study on mechanism. Progress in Modern Biomedicine. 2014;14(18): 3401-4. (in Chinese)
16. Ye DF, Li MF, Zhang YH, Wang XH, Liu H, Wu WT, et al. Cryptotanshinone regulates androgen synthesis through the ERK/c-Fos/CYP17 pathway in porcine granulosa cells. Evid Based Complement Alternat Med. 2017;2017:5985703.
17. Canfield SG, Stebbins MJ, Morales BS, Asai SW, Vatine GD, Svendsen CN, et al. An isogenic blood-brain barrier model comprising brain endothelial cells astrocytes and neurons derived from human induced pluripotent stem cells. J Neurochem. 2017;140(6):874-88.
18. Thao LQ, Byeon HJ, Lee CK, Lee SH, Lee ES, Choi HG, et al. Pharmaceutical potential of tacrolimus-loaded albumin nanoparticles having targetability to rheumatoid arthritis tissues. Int J Pharm. 2016;497(1–2):268-76.
19. Wan X, Zheng XY, Pang XY, Pang ZQ, Zhao JJ, Zhang ZM, et al. Lapatinib-loaded human serum albumin nanoparticles for the prevention and treatment of triple-negative breast cancer metastasis to the brain. Oncotarget. 2016;7(23):34038-51.
20. Zhang JM, Wang D, Wu YH, Li W, Hu YC, Zhao G, et al. Lipid-polymer hybrid nanoparticles for oral delivery of tartary buckwheat flavonoids. J Agric Food Chem. 2018;66(19):4923-32.
21. Cai H, Wen XX, Wen L, Tirelli N, Zhang X, Zhang Y, et al. Enhanced local bioavailability of single or compound drugs delivery to the inner ear through application of PLGA nanoparticles via round window administration. Int J Nanomedicine. 2014;9:5591-601.
22. Zou L, Wang D, Hu YC, Fu CM, Li W, Dai L, et al. Drug resistance reversal in ovarian cancer cells of paclitaxel and borneol combination therapy mediated by PEG-PAMAM nanoparticles. Oncotarget. 2017;8(36):60453-68.
23. Alshamsan A. Nanoprecipitation is more efficient than emulsion solvent evaporation method to encapsulate cucurbitacin I in PLGA nanoparticles. Saudi Pharm J. 2014;22(3): 219-22.
24. Lewis DI. Animal experimentation: implementation and application of the 3Rs. Emerg top life sci. 2019;3(6):675-9.

วารสารการแพทย์แผนจีนในประเทศไทย
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นิพนธ์ต้นฉบับ

การเตรียมสารกลุ่มแทนชิโนนที่บรรจุใน PLGA/NPs และผลในการส่งเสริมด้านการเรียนรู้และความจำเมื่อใช้ร่วมกับพิมเสน

หวัง ตี้[1], **หลี่ เวย**[2], **อู๋ อี้หาน**[1], **จาน กู้**[1], **จาง เฉิน**[1], **เกา ฮุ่ยเล่อ**[3],**โจว เลี่ยง**[4], **จาง จินหมิง**[1]

[1]*ห้องปฏิบัติการหลักประจำมณฑลด้านทรัพยากรยาแผนจีนภาคตะวันตกเฉียงใต้ มหาวิทยาลัยการแพทย์แผนจีนเฉิงตู*

[2]*วิทยาลัยเวชศาสตร์พื้นฐาน มหาวิทยาลัยเฉิงตู*

[3]*วิทยาลัยเภสัชกรรมแผนจีนและตะวันตก มหาวิทยาลัยซื่อชวน*

[4]*ห้องปฏิบัติการหลักด้านการแปรรูปธัญพืช กระทรวงเกษตรและชนบท มหาวิทยาลัยเฉิงตู*

บทคัดย่อ: ความบกพร่องด้านการเรียนรู้และความจำเป็นอาการที่พบได้บ่อยที่สุดโดยมีสาเหตุมาจากโรคทางระบบประสาทส่วนกลาง ซึ่งนำไปสู่คุณภาพชีวิตที่แย่ลง ถึงอย่างไรก็ตามการรักษาด้วยยาที่ใช้กันโดยทั่วไปนั้นให้ผลการรักษายังไม่เป็นที่น่าพอใจ อีกทั้งยังไม่ทราบกลไกที่แน่ชัดของการเกิดภาวะดังกล่าว รวมถึงความท้าทายในการนำส่งยาผ่านชั้นเยื่อบุที่เป็นแนวกั้นกรองทางธรรมชาติระหว่างหลอดเลือดฝอยในสมองและเซลล์สมองรวมถึงส่วนอื่นๆ ที่ประกอบเป็นเนื้อเยื่อสมอง วัตถุประสงค์ในการศึกษานี้เป็นการพัฒนาวิธีการรักษาโดยใช้สารกลุ่มแทนชิโนนที่ถูกบรรจุไว้ในอนุภาคนาโนร่วมกับการใช้พิมเสนเพื่อทำให้การเรียนรู้และความจำนั้นดียิ่งขึ้น วิธีที่ใช้ในเบื้องต้นโดยนำสารสำคัญคือ แทนชิโนน IIA (TanIIA) และคริปโตแทนชิโนน (CTan) ซึ่งสกัดได้มาจากพืชที่มีชื่อวิทยาศาสตร์ว่า *Salvia miltiorrhiza* Bunge ซึ่งสารสำคัญทั้งสองชนิดได้ถูกนำไปบรรจุไว้ในโคโพลีเมอร์ PLAG ได้เป็นอนุภาคนาโนที่เรียกว่า TC@PLGA/NPs โดยใช้เทคนิคการระเหยด้วยตัวทำละลายขั้นตอนเดียว ประสิทธิภาพการห่อหุ้ม TanIIA และ CTan ของ TC@PLGA/NPs มีค่าเท่ากับ 85.31±1.28% และ 86.42±2.07% ตามลำดับ ค่า LE% ของ TanIIA และ CTan มีค่าเท่ากับ 1.24±0.09% และ 1.53±0.15% ตามลำดับ มีขนาดของอนุภาคโดยเฉลี่ยเท่ากับ 194.2±3.5 นาโนเมตร และมีความคงสภาพของการกักเก็บรักษาอยู่ในระดับที่ดี ผลของการวิเคราะห์เชิงความร้อนด้วยเทคนิคดิฟเฟอร์เรนเชียลสแกนนิ่งคาลอรี่เมทรี (DSC) พบว่าสารกลุ่มแทนชิโนนที่ถูกห่อหุ้มในโพลีเมอร์ PLAG ได้ดีไม่ใช่เป็นการผสมกันทางกายภาพ ส่วนการทดลองทางพฤติกรรมโดยใช้วิธี The Morris water maze (MWM) แสดงให้เห็นว่าการให้ TC@PLGA/NPs ร่วมกับพิมเสน (BNL+TC@PLGA/NPs) โดยฉีดเข้าทางหลอดเลือดดำ สามารถเพิ่มความสามารถด้านการเรียนรู้และความจำเชิงพื้นที่ของหนูทดลองที่เป็นโรคอัลไซเมอร์เมื่อเปรียบเทียบกับกลุ่มควบคุม โดยการให้พิมเสนร่วมกับ TC@PLGA/NPs สามารถลดระยะเวลาแฝงของการหลบหนีและระยะทางในการว่ายน้ำของหนูที่มีภาวะสมองเสื่อมที่ชักนำด้วยสาร scopolamine อย่างมีนัยสำคัญ ขณะเดียวกันยังลดการแสดงออกที่เกิดจากสารมาลอนไดอัลดีไฮด์ (MDA) และเอนไซม์แอซีทิลโคลีนเอสเทอเรส (AchE) ในสมองส่วนฮิปโปแคมปัสอีกด้วย บทสรุปคือข้อได้เปรียบจากการใช้พิมเสนที่ช่วยในการลำเลียงยาผ่านเยื่อบุแนวกั้นระหว่างหลอดเลือดฝอยและเนื้อเยื่อสมองกับการใช้สารกลุ่มแทนชิโนนที่ถูกบรรจุไว้อนุภาคนาโนร่วมกัน ทำให้ยกระดับความสามารถในการเรียนรู้และความจำในหนูทดลองให้สูงขึ้นอย่างมีนัยสำคัญ ผลการศึกษาวิจัยนี้ได้ให้แนวทางที่มีประสิทธิผลในการรักษาโรคทางระบบประสาทส่วนกลาง

คำสำคัญ: แทนชิโนน; คริปโตแทนชิโนน; พิมเสน; ความบกพร่องด้านการเรียนรู้และความจำ; PLGA

ผู้รับผิดชอบบทความ: โจว เลี่ยง: zouliangcdu@126.com;
จาง จินหมิง: cdutcmzjm@126.com

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原创论文

丹参酮包封 PLGA 纳米粒的制备及其与冰片联合对认知改善的研究

王迪[1]，李维[2]，吴亿晗[1]，詹固[1]，张晨[1]，高会乐[3]，邹亮[4]，章津铭[1]

[1]*成都中医药大学药学院西南特色中药资源国家重点实验室*

[2]*成都大学基础医学院*

[3]*四川大学华西药学院*

[4]*成都大学农业农村部杂粮加工重点实验室*

摘要：认知功能障碍是中枢神经系统疾病引起的最常见的症状，会导致患者生活质量下降。然而，由于其发病机制不明确以及血脑屏障给药带来的挑战，常用的治疗药物几乎没有表现出令人满意的临床效果。本文的旨在开发一种丹参有效组分与冰片共载纳米粒的治疗方法，从而改善认知功能障碍。首先采用一步溶剂蒸发法将丹参酮 IIA（TanIIA）和隐丹参酮（CTan）这两种丹参的有效组分载于PLGA聚合物材料中，得到丹参有效组分共载聚合物纳米粒（TC@PLGA/NPs）。在对 TC@PLGA/NPs 进行优化表征的基础上，采用冰片联合给药对 Morris 水迷宫大鼠空间记忆的影响进行探究。本研究结果显示 TC@PLGA/NPs 中 TanIIA 和的 CTan 包封率分别为 85.31±1.28%、86.42±2.07%，载药量分别为 1.24±0.09%、1.53±0.15%。平均纳米粒径为 194.2±3.5 nm，贮存稳定性好。差式扫描量热分析结果表明，丹参有效组分能够较好地包载于 PLGA 聚合物中，并不是物理混合。水迷宫行为学实验表明，与其他对照组相比，静脉给药 TC@PLGA/NPs 联合冰片（BNL+TC@PLGA/NPs）可显著提高阿尔茨海默病（AD）模型大鼠的空间学习记忆能力。其中，BNL+TC@PLGA/NPs 可显著缩短东莨菪碱诱导痴呆大鼠的逃避潜伏期和游泳距离，同时降低海马组织中丙二醛（MDA）和乙酰胆碱酯酶（AchE）的表达。本研究表明将冰片增强血脑屏障药物传递的优势与丹参有效组分共载纳米粒相结合，可显著提高大鼠的学习能力和认知功能。这将为中枢神经系统疾病的治疗提供有效策略。

关键词：丹参酮IIA；隐丹参酮；冰片；认知障碍；PLGA

通讯作者：邹亮：zouliangcdu@126.com；
章津铭：cdutcmzjm@126.com