Lactoferrin modification of berberine nanoliposomes enhances the neuroprotective effects in a mouse model of Alzheimer’s disease

Lin Wang1-8, Bi-Qiang Zhou1-4, Ying-Hong Li1, Qian-Qian Jiang1, Wei-Hong Cong1, Ke-Ji Chen1,*, Xiao-Min Wen4,*, Zheng-Zhi Wu1,*

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From the Contents

Introduction ........................................ 226
Materials and methods ................................ 227
Results ............................................... 229
Discussion ......................................... 229

Abstract

Previous studies have shown that berberine has neuroprotective effects against Alzheimer’s disease, including antagonizing tau phosphorylation, and inhibiting acetylcholinesterase activity and neural cell apoptosis. However, its low bioavailability and adverse reactions with conventional administration limit its clinical application. In this study, we prepared berberine nanoliposomes using liposomes characterized by low toxicity, high entrapment efficiency, and biodegradability, and modified them with lactoferrin. Lactoferrin-modified berberine nanoliposomes had uniform particle size and high entrapment efficiency. We used the lactoferrin-modified berberine nanoliposomes to treat a mouse model of Alzheimer’s disease established by injection of amyloid-beta 1–42 into the lateral ventricle. Lactoferrin-modified berberine nanoliposomes inhibited acetylcholinesterase activity and apoptosis in the hippocampus, reduced tau over-phosphorylation in the cerebral cortex, and improved mouse behavior. These findings suggest that modification with lactoferrin can enhance the neuroprotective effects of berberine nanoliposomes in Alzheimer’s disease.

Key Words: acetylcholinesterase; Alzheimer’s disease; apoptosis; berberine; brain targeting; lactoferrin; nanoliposomes; neuroprotective effects; tau phosphorylation

Introduction

Alzheimer’s disease (AD) is a common neurodegenerative disorder, accounting for 60–80% of dementia cases worldwide, and its clinical symptoms mainly include progressive cognitive impairment, mnemonic trouble, language barriers, and personality changes (Gopalans et al., 2020). The available clinical drugs for AD are mainly acetylcholinesterase (AChE) inhibitors and glutamate receptor antagonists, which are only capable of relieving temporary clinical symptoms and fail to postpone or block the pathological course of AD. Furthermore, patients subjected to prolonged medication with these drugs often have difficulty tolerating them because of toxic side effects (Wang et al., 2018; Cummings et al., 2020). Therefore, there is an urgent need to improve conventional anti-AD drugs and develop new drugs for AD (Ballard et al., 2018; Cummings et al., 2020). Therefore, it is vital to use an effective drug carrier to minimize these limiting factors and enhance the efficacy of berberine (Raju et al., 2019).

Berberine is a natural active ingredient extracted from rhizoma of Coptis chinensis. Its pharmacological action on the nervous system was initially reported in 1970 (Shanbhag et al., 1970). Evidence strongly supports that berberine has neuroprotective and substantial multi-target anti-AD pharmacodynamic effects, such as inhibiting AChE activity, antagonizing p-tau, and reducing neural cell apoptosis (Bahrami-Madiseh et al., 2017; Yuan et al., 2019; Imenshahidi and Hosseinzadeh, 2020). By reducing glycogen synthase kinase-3β activity and elevating protein phosphatase 2A activity in neurons, berberine inhibits tau over-phosphorylation at multiple sites and reduces neurofibrillary tangles to protect neural cells in the brain (Gong et al., 2006; Durairajan et al., 2012; Zhang et al., 2018). Berberine inhibits AChE, malondialdehyde, protein carbonyl activity, and DNA cleavage in hippocampus, increases anti-oxidative capacity, and improves spatial recognition memory in a mouse model of AD (de Oliveira et al., 2016; Sadraie et al., 2019). However, low bioavailability of berberine makes it difficult for a routine oral formulation to reach the effective drug concentration, and berberine’s serious adverse reactions greatly impede its use for clinical application (Mirhadi et al., 2018; Song et al., 2020). Therefore, it is vital to use an effective drug carrier to minimize these limiting factors and enhance the efficacy of berberine (Raju et al., 2019).

Liposomes are a desirable drug carrier characterized by low toxicity, high entrapment efficiency, and biodegradability; furthermore, surface modification with polyethylene glycol can prolong the in vivo circulation duration of the drug (Pattini et al., 2015; Cai et al., 2022; Hernandez and Shukla, 2022). Wang et al. (2017) reported that compared with berberine solution, berberine liposomes were available at an appropriately high drug concentration to more efficiently inhibit tumor growth in mice. Allijn et al. (2017) demonstrated that entrapment with liposomes enabled high solubility of berberine, which effectively alleviated post-infarction cardiac insufficiency in mice. Calvo et al. (2020) demonstrated that use of berberine liposomes in visceral leishmaniasis prevented rapid hepatic metabolism of berberine and improved selective drug delivery to the infected organs. To the best of our knowledge, use of berberine liposomes in the prevention and treatment of AD has not yet been reported.

Graphical Abstract

Lactoferrin modification of berberine nanoliposomes reduces Alzheimer’s-like amyloid-β neurotoxicity through inhibiting AChE activity, Tau over-phosphorylation, and apoptosis

*These two authors contributed equally to this paper.

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A key issue of AD prevention and treatment with berberine liposomes is how to increase drug concentration at the lesion site in the brain. Modification with lactoferin (Lf) is likely to solve this issue. Blood-brain barrier (BBB) endothelial cells express Lf receptors (LfR). If binding to LfR can facilitate an Lf-modified drug carrier system through the BBB to increase drug concentration in the brain, and Lf has been widely applied in targeted nanotherapy for nervous system disorders (Huang et al., 2007; Yan et al., 2018; Mittal et al., 2020).

Liu et al. (2017) found that Lf-modified daunorubicin and magnolol nanoliposomes were transported via the BBB and promoted drug accumulation in brain tumor tissue. Karami et al. (2019) showed that Lf-modified indinavir nanosomes enhanced the brain permeation of Indinavir remarkably. Huang et al. (2013) reported that Lf modification of 99mTc labeled N,N-bis(2-mercaptoethyl)-N′,N′-diethylthiacyanine increased the uptake of nanoemulsion by brain tumor tissue. Karami et al. (2019) showed that Lf-modified Indinavir nanoemulsion facilitated the brain permeation of Indinavir remarkably. Therefore, Lf modified DiR PEGylated nanoliposomes with brain-targeting function. The neuroprotective mechanism of berberine nanoliposomes on the behavioral capacity of a mouse model of AD-like amyloid β (Aβ) neurotoxicity was explored.

Methods

Preparation of BR nanoliposomes

Preparation of blank nanoliposomes

The blank nanoliposomes were produced using modified ethanol (Ante Biochemistry Co., Ltd., Suzhou, Anhui Province, China) injection. The modified ethanol was mixed with ethanol 10% (v/v) and then heated in a water bath at 65°C. (NH₄)₂SO₄ solution preheated to the same temperature was added to each vial, which was then stirred in a water bath for 20 minutes. The preliminary products were further processed by an ultrasonic cell disruptor (YJY-2, Scientz Biotechnology Co., Ltd., Ningbo, Zhejiang Province, China) and granulated by passing through 0.8, 0.45, and 0.22 μm microporous filters successively, resulting in suspensions of blank nanoliposomes. The blank nanoliposomes (PL) and carboxyl-containing PEGylated blank nanoliposomes (PL-CHOH), respectively, were mixed with ethanol 10% (v/v) and then heated in a water bath at 65°C. The ethanol solution was loaded into the top of a G100 column (Yuanye Biological Technology Co., Ltd., Shanghai, China) and centrifuged and eluted three times. The eluate was combined and mixed well to form a liposome suspension having an (NH₄)₂SO₄ concentration of 0.25 M. Desired amounts of liposomes were prepared by adding water to the liposome suspension. The solution was then incubated at 37°C for 120 hours and kept for later use. Each sample was diluted with methanol to 10 mL to disrupt the nanoliposomes, and then its absorbance at 420 nm was determined using a UV spectrophotometer (UV100, Wanyi Science and Technology Co., Ltd., Heifei, Anhui Province, China), denoted as A0.

Preparation of BR-CL, BR-PL, and BR-PL-CHOH

The blank nanoliposomes suspension was loaded into the top of a G100 column (Yuanye Biological Technology Co., Ltd., Shanghai, China) and centrifuged at 1500 × g for 4 minutes. The preliminary products were further processed by an ultrasonic cell disruptor (YJY-2, Scientz Biotechnology Co., Ltd., Ningbo, Zhejiang Province, China) and granulated by passing through 0.8, 0.45, and 0.22 μm microporous filters successively, resulting in suspensions of blank nanoliposomes. The blank nanoliposomes (PL) and carboxyl-containing PEGylated blank nanoliposomes (PL-CHOH), respectively, were mixed with ethanol 10% (v/v) and then heated in a water bath at 65°C. The ethanol solution was loaded into the top of a G100 column, and centrifuged at 1500 × g for 4 minutes. Double-distilled water was added, and the resulting mixture was centrifuged at 1500 × g for 4 minutes. This step was repeated, and eluted water was added. Eluates were diluted with methanol to 10 mL to disrupt the liposomes. UV spectrophotometry was performed. The absorbance value was recorded as Alp. BR entrapment efficiency was calculated using the following formula:

$$\text{EE} = \frac{A_{1} - A_{2}}{A_{1}} \times 100\% \tag{1}$$

Grafting ratio of Lf in BR-Lf

Lf grafting ratio was determined by Coomassie brilliant blue method (Han et al., 2010). BR-Lf reaction solution (0.1 mL) was collected in duplicate. One sample was passed through dextran gel Sephadex G-100 column to remove unreacted Lf, and the second sample did not pass through the column. Specifically, 5 mL of acidic Coomassie brilliant blue G250 chromatography solution was added to 0.1 mL of BR-Lf. Absorbances A1 and A2 at 595 nm were determined using a UV spectrophotometer. Lf grafting ratio was calculated using the following formula:

$$\text{Lf grafting ratio} = \frac{A_{2} - A_{1}}{A_{2}} \times 100\% \tag{2}$$

Establishment of a mouse model of AD-like Aβ neurotoxicity

Ninety-nine male healthy ICR mice (23–25 g, 2 months old, Liaoning Changsheng Biotechnology Ltd., Benxi, Liaoning Province, China, license No. SCXK (Lia) 2020-0001) were maintained at 25 ± 1°C in a 12-hour dark/light cycle with free access to food and drinking water. Mice were housed in this study. This experiment was approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University. On August 24, 2020 (approval No. SYPH-IACUC-C2020-8-24-121). All experiments were designed and reported in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (Percie du Sert et al., 2010).

To perform behavioral tests and to investigate the mechanisms underlying neuroprotective effects, 54 mice were randomly divided into six groups (n = 9 per group): sham control (vehicle-injected, model (Aβ-injected), BR solution (AB + BR-S), BR-CL (AB + BR-CL), BR-PL (AB + BR-PL), and BR-Lf (AB + BR-Lf) groups. To perform fluorescence imaging, 45 mice were randomly divided into three groups (n = 15 per group): DiR-CL (DiR + BR-CL), DiR-PL (DiR + BR-PL), and DiR-Lf (DiR + BR-Lf) groups. The mouse model of AD-like Aβ neurotoxicity was established by injection of Aβ-protein fragment 1–42 (AB42) into the lateral ventricle (Goswami et al., 2020). AB42 (0.1 mg; Sigma-Aldrich, St. Louis, MO, USA) was dissolved with 12 μL of dimethyl sulfoxide. Then, 161 μL of normal saline was added and the solution was incubated at 37°C for 120 hours and kept for later use. Each mouse was anesthetized with 2.5% avertin (240 mg/kg; Sigma-Aldrich) by intraperitoneal injection and then injected with the Aβ solution. After 4 hours, mice were anesthetized and sacrificed to collect brain tissue.
intraparenchymal injection. Fur was removed from the cranial incision region, the cranial skin was disinfected with alcohol and incised, and the skull was fixed to a brain stereotaxic apparatus (NARISHIGE SR-5N, Tokyo, Japan). The position of the needle entry point was determined according to a mouse brain stereotaxic map (left lateral ventricle: 0.5 mm away from the back of anterior fontanel, 1.1 mm left of the sagittal suture, and 3.0 mm below the skull surface) (Ji et al., 2014). Then, 3 μL of Aβ1-42 solution (containing 410 pmol Aβ1-42, 0.1 mL vehicle for sham group) was perpendicularly injected with a microinjection (Hamilton, Bonaduz, Switzerland) using a 3-minute slow push followed by a 3-minute indwelling. Benzylpenicillin sodium was injected for anti-infection treatment.

Fluorescence imaging

Fluorescence imaging was used to observe the distribution of different DiR nanoliposomes in the model mice and evaluate their brain targeting. Imaging was performed after the models were established 2 days. The mice received a single injection of DIR-CL, DI-PL, or DIR-Lf at a dose of 2.0 mg/kg (based on DiR) via the caudal vein. At 2, 4, 8, 12, and 24 hours after the injection, the mice were euthanized with an overdose of injection in vivo small animal imaging system (Bruker, Billerica, MA, USA), and both fluorescence and white light images were taken. After photography at each time point, three mice were sacrificed by cervical dislocation, and the brains, hearts, lungs, livers, spleens, and kidneys were then isolated and photographed under a fluorescence microscope. The molecular imaging software supplied with the system was used to quantitatively analyze near-infrared fluorescence intensity of the brain ex vivo.

Behavioral capacity tests

Different BR preparations (10 mg/kg) or vehicle (normal saline, 0.1 mL/10 g) were injected into mice by intravenous injection once every other day, starting on the day of the Aβ injection, for a total of seven doses.

The Y-maze test

To assess the effects of different BR preparations on spatial recognition memory, a Y-maze test was performed after the Y-maze test apparatus (made by Shenyang Pharmaceutical University) was composed of three arms at an angle of 120° between adjacent arms, referred to as arms A–C. After completion of adaptive training, each animal was placed at the end of arm A and was allowed to visit the three arms freely. The total number of entries in the three arms within 5 minutes (N) and the sequence of arm entries were recorded. Successive entries in three different arms were counted as one correct alternation, and the number of correct alternations (Nc) was recorded. Throughout the testing, excreta were cleaned in time to eliminate remaining odor. Spontaneous alternation behavior was used to assess spatial working memory capacity of each mouse and was calculated using the following formula:

\[
\text{Alternation behavior} (\%) = \frac{N_c}{N-2} \times 100\%
\]  

Novel object recognition test

To assess the effects of different BR preparations on learning and memory ability of mice, we performed a novel object recognition test after the Y-maze test (Jin et al., 2016). Two identical objects (A1 and A2) were placed at the same distance from the margin of an open field. An adapted mouse was put at any position with equal distances from both objects. The time that the mouse explored each object within 5 minutes was recorded. Then, the mouse was returned to its cage. One hour later, either of the objects was replaced by a different object B, and the mouse was put in the apparatus again, and the time that each object was explored within 5 minutes (tB and tA) was recorded. Twenty-four hours later, the object B was replaced by a totally different object C. The number of correct alternations (Nc) was recorded. Throughout the testing, excreta were cleaned in time to eliminate remaining odor. Spontaneous alternation behavior was used to assess spatial working memory capacity of each mouse and was calculated using the following formulas, respectively:

\[
\text{Preferential index (1 h)} = \frac{t_B}{t_A} \times 100\%
\]

\[
\text{Preferential index (24 h)} = \frac{t_C}{t_A} \times 100\%
\]

Exploration into mechanism of neuroprotective effects

Harvesting

After the behavioral tests, the mice were put into a closed glass container with 4% ether solution for anesthesia. Four mice in each group were perfused to harvest their brains. The brains were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer saline (pH 7.4) in vivo for 24 hours, then sections were cut into 5-μm-thick sections and embedded in paraffin. The sections were dewaxed and hydrated. After antigen retrieval with citrate, endogenous peroxidases were blocked with hydrogen peroxide. Then, the sections were incubated with 5% Blocking serum albumin (BSA) for 1 hour, and were mounted on slides. Under a microscope (DS-R2, Nikon Corporation, Tokyo, Japan), the region of interest was examined and labeled cells per mm² were analyzed by ImageJ software (1.8.0; National Institutes of Health, Bethesda, MD, USA) (Schneider et al., 2012).

AchE activity in mouse hippocampal tissue

Frozen hippocampal tissue from each group (n = 4 per group) was collected and tissue lysis buffer (5 μL of phenylmethylsulfonyl fluoride) was added. The hippocampus and cortex were isolated and transferred into an epoxy tube, which was then quickly frozen in liquid nitrogen and stored at –80°C. The frozen hippocampal tissue was selected at random for use in experiments.

AchE activity in mouse hippocampal tissue

Fluorescence imaging was used to observe the distribution of different DiR nanoliposomes in the model mice and evaluate their brain targeting. Imaging was performed after the models were established 2 days. The mice received a single injection of DIR-CL, DI-PL, or DIR-Lf at a dose of 2.0 mg/kg (based on DiR) via the caudal vein. At 2, 4, 8, 12, and 24 hours after the injection, the mice were euthanized with an overdose of injection in vivo small animal imaging system (Bruker, Billerica, MA, USA), and both fluorescence and white light images were taken. After photography at each time point, three mice were sacrificed by cervical dislocation, and the brains, hearts, lungs, livers, spleens, and kidneys were then isolated and photographed under a fluorescence microscope. The molecular imaging software supplied with the system was used to quantitatively analyze near-infrared fluorescence intensity of the brain ex vivo.

Fluorescence imaging was used to observe the distribution of different DiR nanoliposomes in the model mice and evaluate their brain targeting. Imaging was performed after the models were established 2 days. The mice received a single injection of DIR-CL, DI-PL, or DIR-Lf at a dose of 2.0 mg/kg (based on DiR) via the caudal vein. At 2, 4, 8, 12, and 24 hours after the injection, the mice were euthanized with an overdose of injection in vivo small animal imaging system (Bruker, Billerica, MA, USA), and both fluorescence and white light images were taken. After photography at each time point, three mice were sacrificed by cervical dislocation, and the brains, hearts, lungs, livers, spleens, and kidneys were then isolated and photographed under a fluorescence microscope. The molecular imaging software supplied with the system was used to quantitatively analyze near-infrared fluorescence intensity of the brain ex vivo.
Results

Characterization of BR nanoliposomes

Table 1 shows that the BR-CL, BR-PL, and BR-Lf nanoliposomes particle sizes were similar and narrowly distributed. The entrapment efficiency of BR nanoliposomes was more than 90%. The grafting ratio of Lf in BR-Lf was 65.9 ± 2.2% as shown in Table 1. Lf modification did not have much impact on particle size or entrapment efficiency of BR nanoliposomes. Microcrystalline observation showed that BR-CL, BR-PL, and BR-Lf were all quasi-circular unilamellar nanoliposomes with evident bilayer membranes (Figure 1). They were evenly distributed and their sizes were consistent with the particle size analysis.

Table 1 | Particle size, PI, EE and Lf grafting ratio of BR nanoliposomes and DiR nanoliposomes

| BR-CL | BR-PL | BR-Lf | DiR-CL | DiR-PL | DiR-Lf |
|-------|-------|-------|--------|--------|--------|
| Particle size (nm) | 129.5 ± 4.6 | 100.4 ± 3.2 | 118.6 ± 4.5 | 128.7 ± 3.9 | 120.6 ± 3.5 |
| PI (1 h) | 0.235 ± 0.012 | 0.216 ± 0.015 | 0.246 ± 0.017 | 0.207 ± 0.014 | 0.228 ± 0.013 |
| EE (%) | 92.13 ± 0.0 | 94.72 ± 0.4 | 90.41 ± 0.2 | 95.84 ± 0.2 | 96.52 ± 0.9 |
| Lf grafted (%) | 65.9 ± 2.2 | 65.9 ± 2.2 | 65.9 ± 2.2 | 65.9 ± 2.2 | 66.2 ± 3.8 |

 LF improves brain targeting of BR nanoliposomes

In vivo fluorescence imaging was used to investigate the effect of Lf modification on the brain targeting capacity of BR nanoliposomes. During a 24-hour test period, fluorescence signals in the head, liver, and lung of mice in each liposome group initially increased and then decreased over time (Figure 2A). In the DiR-CL group, a high fluorescence signal in the liver and a weaker fluorescence signal in the head were observed just 2 hours after injection. In the DiR-PL group, a weak fluorescence signal was present in the brain 2 hours after injection, and the signal increased gradually until it reached the maximum at 8 hours. In the DiR-Lf group, the fluorescence signal in the brain was significantly higher than those in the DiR-CL and DiR-PL groups, and the high fluorescence signal was observed in the brain 2 hours after injection, indicating that the drug had reached the brain.

To confirm the in vivo imaging findings, we performed ex vivo mouse organ imaging (Figure 2B and C). Statistical analysis showed that there were significant differences between different preparations at the same time point and in the same preparation between different time points. The groups ordered by relative fluorescence intensity at each time point were DiR-Lf > DiR-PL > DiR-CL (P < 0.01), which was consistent with the in vivo imaging findings. The maximum fluorescence intensity of the DiR-Lf group at 4 hours was significantly higher than that of the DiR-PL group at 8 hours (P < 0.01).

BR-Lf improves behavioral capacity of a mouse model of AD-like Aβ neurotoxicity

The in vivo Y-maze test was used to determine the effects of different BR formulations on spatial recognition memory of the mouse model of AD-like Aβ neurotoxicity (Figure 3A). Compared with the sham group, the Aβ-injected model group had significantly reduced spontaneous alternation behavior (P < 0.01), suggesting that Aβ injection induces working memory impairment and spatial memory impairment in mice. Spontaneous alternation behavior increased in the BR-S group and in the three different BR nanoliposomes groups compared with that in the model group. Previous studies have reported that LfR increased in the brain of people with neurodegenerative disorders, and have demonstrated that Lf-modified PEGylated liposomes bind to LfR and are converted into positively-charged groups, which bind to the negatively-charged BBB under physiological conditions (Suzuki et al., 2005; Agrawal et al., 2017). LfR mediate specific one-way transport of Lf-carried drugs through differentiated BBB under physiological conditions (Suzuki et al., 2005; Agrawal et al., 2017). Lf modification enables accumulation of the carried drug in brain cells in vivo, because of ease in operation and rapid measurement (Lai et al., 2015; Dou et al., 2019). Lf modification enables accumulation of the carried drug in brain tissue, as detected by high fluorescence intensity and long residence time, in in vivo imaging studies, suggesting good brain-targeting specificity (Meng et al., 2018; Zhao et al., 2018). In this study, the fluorescence intensity of DiR-Lf was significantly increased compared with that of DiR-CL and DiR-PL in the brain, suggesting that DiR-Lf was transported across the BBB by the LfR, which greatly increases the brain-targeting capacity of the liposomes.

Discussion

In vivo fluorescence imaging has been increasingly applied in AD diagnosis and treatment. Studies in in vivo and post-mortem studies of anti-Aβ drugs in AD mice have demonstrated that Lf modification increases the brain uptake of the Lf-modified Aβ peptide, probably because favorable entropy increment promoted BR binding to AChE, and interaction between two types of molecules coupled with slight change in protein conformation led to lower enzymatic activity (Xiang et al., 2009). In a post-mortem study, differences in the dosage form resulted in varied degrees of AChE inhibition; entrapment of BR by Lf-modified PEGylated liposomes greatly increased the concentration of BR reaching the brain, and an Lf-modified polymer or lipid drug carrier enhanced a significant rise in BBB permeability of a non-neuroactive drug (Moch et al., 2023), markedly promoting drug uptake in the brain (Gothwal et al., 2019). In the present study, BR-Lf provided the strongest AChE inhibition of all the nanoliposomes at the same dose.
Effects of different BR formulations on p-tau level in cerebral cortex of a Sham Model. BR-S: BR solution; BR-CL: BR common nanoliposomes; BR-PL: BR PEGylated nanoliposomes; BR-Lf: BR PEGylated nanoliposomes with lactoferrin modified.

Distribution of different DiR nanoliposomes in AD-like Aβ neurotoxicity model mice at different time points. (A) In vivo (A) and ex vivo (B) fluorescence images in the brain of a mouse model of AD-like Aβ neurotoxicity. Fluorescence signal in the DiR-Lf group was significantly higher than that in the DiR-CL and DiR-PL groups. There was significant difference in fluorescence intensity between the DiR-CL, DiR-PL and DiR-Lf groups. (C) Ex vivo fluorescence intensity of different DiR nanoliposomes in the brain of a mouse model of AD-like Aβ neurotoxicity. Data are expressed as the mean ± SD (n = 3). **P < 0.01, vs. DiR-CL group; #P < 0.01, vs. DiR-Lf group; ††P < 0.01 (two-way analysis of variance followed by Duncan’s multiple range test). AD: Alzheimer’s disease; Aβ: amyloid-β protein; DiR: 1,1’-dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine iodide; DiR-CL: DiR common nanoliposomes; DiR-PL (PL): DiR PEGylated nanoliposomes.

Effects of different BR formulations on behavioral capacity tests in a mouse model of AD-like Aβ neurotoxicity. (A) Spontaneous alternation behavior in the Y-maze test. (B, C) Novel object preference index at 1 (B) and 24 (C) hours. Data are expressed as mean ± SD (n = 9). #P < 0.01, vs. sham group; *P < 0.05, **P < 0.01, vs. model group; †P < 0.05, ††P < 0.01 (one-way analysis of variance followed by the least significant difference post hoc test). AD: Alzheimer’s disease; Aβ: amyloid-β protein; BR: berberine hydrochloride; BR-S: BR solution; BR-CL: BR common nanoliposomes; BR-PL: BR PEGylated nanoliposomes; BR-Lf: BR PEGylated nanoliposomes with lactoferrin modified.

Effects of different BR formulations on AChE activity in hippocampal tissue of a mouse model of AD-like Aβ neurotoxicity. Data are expressed as the mean ± SD (n = 4). #P < 0.01, vs. sham group; *P < 0.05, **P < 0.01, vs. model group; †P < 0.05, ††P < 0.01 (one-way analysis of variance followed by the least significant difference post hoc test). AD: Alzheimer’s disease; AChE: acetylcholinesterase; Aβ: amyloid-β protein; BR: berberine hydrochloride; BR-S: BR solution; BR-CL: BR common nanoliposomes; BR-PL: BR PEGylated nanoliposomes; BR-Lf: BR PEGylated nanoliposomes with lactoferrin modified.

Effects of different BR formulations on expression levels of apoptotic proteins Bax and Bcl-2 in hippocampal tissue of a mouse model of AD-like Aβ neurotoxicity. (A) Western blotting of Bax and Bcl-2. β-Actin was an internal reference. (B, C) Quantitative results of relative expression levels of apoptotic proteins Bcl-2 (B) and Bax (C). Data are expressed as the mean ± SD (n = 3). #P < 0.01, vs. sham group; *P < 0.05, **P < 0.01, vs. model group; †P < 0.05, ††P < 0.01 (one-way analysis of variance followed by LSD post hoc test). AD: Alzheimer’s disease; Aβ: amyloid-β protein; BR: berberine hydrochloride; BR-S: BR solution; BR-CL: BR common nanoliposomes; BR-PL: BR PEGylated nanoliposomes; BR-Lf: BR PEGylated nanoliposomes with lactoferrin modified.
Tau is a microtubule-associated protein capable of promoting neuronal microtubule cytoskeleton assembly and maintaining microtubule stability (Duan et al., 2012). In the brain of an AD patient, each tau molecule has two to six more phosphorogenic groups than in people without AD; over-phosphorylation of tau promotes microtubule disintegration, and the microtubules pair with other phosphorylated tau protein molecules to form paired helical filaments that aggregate into neurofibrillary tangles, leading to neuronal apoptosis (Bazragar et al., 2020). In this study, BR-S and BR nanoliposomes inhibited p-tau at Ser396 in the mouse hippocampal tissue to variable extents, perhaps because BR reduced tau over-phosphorylation by activating protein phosphatase 2A and inhibiting activity of protein phosphokinase (Liu et al., 2005; Takashima, 2006). A previous study has proposed that by inhibiting the nuclear factor kappa-B signaling pathway activation and neuroinflammatory responses, BR reduces tau over-phosphorylation to alleviate cognitive symptoms in AD model mice (He et al., 2017). In this study, BR-Lf greatly reduced tau over-phosphorylation at Ser396 and improved working memory and new object recognition in the mouse model of AD-like Aβ neurotoxicity, which is consistent with the above findings.

Anti-apoptotic Bcl-2 and pro-apoptotic Bax are antagonistic proteins; Bcl-2 and Bax can form a dimer to inactivate Bax, and the Bcl-2/Bax ratio is closely related to apoptosis, dictating the level of activation of pro-apoptotic factors caspase-3 (Croker et al., 2011). Caspase-3 is directly involved in the cleavage of Aβ and tau protein and induces apoptosis, and its expression level directly reflects the apoptosis level (Chu et al., 2017). In this study, BR-Lf reversed the changes in Bcl-2 and Bax expression to greatly reduce caspase-3 and caspase-9 activity, suggesting that BR-Lf modulates Bcl-2 and Bax expression to inhibit abnormal apoptosis of hippocampal neurons and alleviate neuronal damage in model mice (Huang et al., 2017). In this apoptosis-inhibiting effect, BR-Lf was superior to BR-S, BR-CL, and BR-PL, indicating that the Lf-modified PEGylated lipid carrier can concentrate BR in the brain and potentiates the neuroprotective activity of BR.

There were some limitations to this study. We did not determine whether the neuroprotective effects of BR-Lf in a mouse model of AD-like Aβ neurotoxicity were dose-dependent. Further, we did not compare between different administration methods. More systematic research is needed, such as investigation of synaptic and postsynaptic markers and gliosis. We will investigate the neuroprotective ability of BR-Lf with different administration methods and doses in future research.

In conclusion, we successfully prepared Lf-modified PEGylated BR nanoliposomes and demonstrated that the modification potentiated the anti-Aβ effect of BR. Lf modification improved the brain-targeting and neuroprotective effects of BR nanoliposomes. BR-Lf improved the behavioral activities and new object recognition in the mouse model of AD-like Aβ neurotoxicity and new object recognition in the mouse model of AD-like Aβ neurotoxicity, which is consistent with the above findings.

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