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Monocyte mediated brain targeting delivery of macromolecular drug for the therapy of depression

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Abstract

Leukocytes can cross intact blood-brain barrier under healthy conditions and in many neurological diseases, including psychiatric diseases. In present study, a cyclic RGD (cRGD) peptide with high affinity for integrin receptors of leukocytes was used to modify liposomes. The cRGD-modified liposomes (cRGDL) showed very high affinity for monocytes *in vitro* and *in vivo* and co-migrated across *in vitro* BBB model with THP-1. The trefoil factor 3 (TFF3), a macromolecular drug, was rapidly and persistently delivered to brain for at least 12 h when loaded into cRGDL while 2.8-fold increase in drug concentration in basolateral amygdala brain regions related to depression was observed. A systemic administration of cRGDL-TFF3 mimicked antidepressant-like effect of direct intra-basolateral amygdala administration of TFF3 solution in rats subjected to chronic mild stress. The effective dual-brain targeting delivery resulting from the combination and co-migration of cRGDL with leukocyte cross BBB may be a promising strategy for targeted brain delivery.

Key words: cRGD; brain-targeting drug delivery system; monocyte; depression; trefoil factor 3

Introduction

Depressive disorder reportedly affects approximately 16% of the population, leading to a major burden on health.[1] Peptide and protein drugs have shown great promise for the treatment of various neuropsychiatric diseases. However, despite their potential, a major challenge is the delivery of peptide and protein drugs across the blood-brain barrier (BBB),[2] which has been considered as the most important barrier that impedes drug transport into the brain via the blood circulation.[3,4] The BBB can exclude from the brain 100% of large-molecule neurotherapeutics and more than 98% of all small-molecule drugs.[4] Many existing peptide and protein drugs are rendered ineffective in the treatment of these clinical disorders because of an inability to effectively deliver and sustain them within the brain.[2] Thus, the need for a brain-targeting drug delivery system is important to effectively treat brain-related diseases.

Trefoil factor 3 (TFF3), a member of the trefoil factor family, is produced by intestinal goblet cells and used for gastrointestinal disease therapy.[5,6] TFF3 injection was recently found to induce Fos-positive cells in magnocellular oxytocin neurons in the hypothalamus, prompting further research on the role of TFF3 in

Abbreviations: cRGD, cyclic RGD; cRGDL, cRGD-modified liposomes; TFF3, trefoil factor 3; cRGDL-TFF3, cRGD liposomes loaded with TFF3; BBB, blood-brain barrier; i.p, intraperitoneal; HBMEC, human brain microvessel endothelial cell; PL, plain liposomes; PL-TTF3, plain liposomes loaded with TFF3; SPC, soybean phosphatidylcholine; EE, entrapment efficiency; UPLC, ultra-performance liquid chromatography; DLS, dynamic light scattering; CFSE, carboxyfluorescein diacetate succinimidyl ester; BLA, basolateral amygdala; OB, olfactory bulbectomy; CMS, chronic mild stress; CNS, central nervous system; VCAM, vascular cell adhesion molecule; ICAM, intercellular adhesion molecule.

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the central nervous system and related disorders.[7] Our recent studies used several behavioral models of depression and suggested that systemic administration (intraperitoneal, i.p.) of high doses of TFF3 produced antidepressant-like effects in both mice and rats.[8] However, this antidepressant-like effect was not observed in depression models when the dosage was reduced to 0.05 mg/kg. Therefore, a high concentration in brain regions related to depression appears to be necessary for the practical application of TFF3.

The neuroinflammation occur under many neurological diseases including depression.[4,9,10] A typical feature of neuroinflammation response is the recruitment of leukocytes (mainly monocytes and neutrophils) to the lesions in brain. Perivascular macrophages, which reside on the parenchymal side of endothelia cells close to astrocyte endfeet, originally derive from circulating phagocytes, such as monocytes, and have shown a remarkable capability to cross an intact BBB, with 80% turnover in 3 months.[11] Thus, one strategy to deliver drugs to the brain under pathological conditions is to exploit these inflammatory cells as targeted delivery systems.

The RGD (Arg-Gly-Asp) peptide can combine with integrin receptors that are expressed on the surface of leukocytes(including monocytes and neutrophils).[12] Liposomes modified with RGD, therefore, may possibly be developed for selective and preferential presentation to blood monocytes/neutrophils. Subsequently, liposomes can be taken into the brain in response to the recruitment of inflammation processes. In our previous study, obvious brain-targeting drug delivery was obtained using RGD liposome.[13]

In the present study, a cyclic RGD (cRGD, Arg-Gly-Asp-D--Phe-Lys) peptide with higher affinity for integrin receptors comparing with RGD[14] was used as a ligand to be coupled with liposomes. Furthermore, the possible mechanisms of brain-targeted delivery were investigated *in vitro* and *in vivo* using a human acute monocytic leukemia cell line (THP-1), human brain microvessel endothelial cell (HBMEC) and inflammation rats. Then, the pharmacological effects of cRGDL loaded with TFF3 (cRGL-TFF3) were studied. The aim of the present study was to verify the brain-targeting effect and potential mechanism of cRGDL and study whether cRGDL enhance the behavioral response to TFF3.

Methods

Animals and cells

Male Sprague-Dawley rats (200-220 g) or male nude mice (20 ± 2 g) were housed under a constant temperature (23 ± 2 °C) and a 12 h/12 h light/dark cycle with free access to food and water. All of the animal experiments were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the procedures were approved by the Peking University Animal Use Committee (LA2012/21) or by the Ethics Committee of Fudan University (SYXK-2010-0099).

THP-1 cell line was obtained from the Institute of Biochemistry and Cell Biology (Shanghai, China) and cultured as previously described.[15] HBMEC was from ScienCell (Carlsbad, CA, USA) and cultured on upper chamber of Transwell insert of purified collagen with endothelial cell medium.[16,17]

Liposome preparation

Plain liposomes (PL) loaded with TFF3 (PL-TTF3) were formulated with soybean phosphatidylcholine (SPC), DSPE-PEG (PEG 2000), DSPG (Merck, Schaffhausen, Switzerland) and cholesterol and were prepared using a modified reverse-phase evaporation method described previously.[18] Briefly, A mixture of phospholipids and cholesterol,[19] including DSPE-PEG 2mol% of total lipid, was dissolved in 15 ml chloroform and 5 ml phosphate buffer (pH 7.4) containing TFF3 (10:1 lipid:drug molar ratio, Beijing Yong Kang Jia Xin Science and Technology Development, Beijing, China). The resulting two-phase system was subjected to bath-type sonication and then was placed in a rotary evaporator under reduced pressure for at least 12 h until a homogeneous suspension of PL-TFF3 was obtained.

For the preparation of cRGDL-TFF3, 2mol% cRGD-PEG-DSPE (GL Biochem, Shanghai, China) was used in the lipid formulation instead of DSPE-PEG.[13]

For the preparation of fluorescence PL or cRGDL, coumarin 6, TFF3-Cy5 or TFF3-Cy7 was loaded into liposomes to obtain C6-PL, C6-cRGDL; PL-TFF3-Cy5, cRGDL-TFF3-Cy5; PL-TFF3-Cy7 and cRGDL-TFF3-Cy7. C6-PL non-DSPG and C6-cRGDL non-DSPG present the lipid formulation without the addition of DSPG. For the preparation of liposomes of various particle sizes, different ultrasonic times were used. Finally, C6-loaded liposomes of different particle size (300, 200, 100, and 50 nm) were obtained for further study.

Liposome characterization evaluation

The entrapment efficiency (EE) was determined by size exclusion chromatography and ultra-performance liquid chromatography (UPLC) method. The equation for calculating EE was the following:

$$EE = W_{interior}/W_{total} \times 100\%$$

$W_{interior}$ represents the intraliposomal content of TFF3, and W_{total} represents the total content in the liposomal suspension when Triton-100 was added to the suspension. The mean diameters and zeta potential of PL-TFF3 and cRGDL-TFF3 were determined by dynamic light scattering (DLS) using a ZetaSizer Nano series 3600 (Malvern, GmbH, Herrenberg, Germany).

Uptake and competition assays of liposomes on leukocytes in vitro

THP-1 cells were used to investigate the possible mechanism of the uptake of liposomes by leukocytes. C6-PL or C6-cRGDL with different lipid concentrations (0.66, 1.32, and 1.97 μmol lipid/L) was added to THP-1 cells for 1h incubation at 37 °C. After incubation, the liposome suspension was removed, and the cells were washed three times with PBS by centrifugation. The cells were re-suspended and visualized under an IX2-RFACA fluorescent microscope (Olympus, Osaka, Japan). The fluorescence intensity of THP-1 of C6-PL group and C6-cRGDL group