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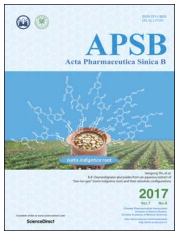

ORIGINAL ARTICLE

Surface modification of PGP for a neutrophil–nanoparticle co-vehicle to enhance the anti-depressant effect of baicalein

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Abstract Exploiting cells as vehicles for nanoparticles combined with therapy has attracted increasing attention in the world recently. Red blood cells, leukocytes and stem cells have been used for tumor immunotherapy, tissue regeneration and inflammatory disorders, and it is known that neutrophils can accumulate in brain lesions in many brain diseases including depression. *N*-Acetyl Pro–Gly–Pro (PGP) peptide shows high specific binding affinity to neutrophils through the CXCR2 receptor. In this study, PGP was used to modify baicalein-loaded solid lipid nanoparticles (PGP-SLNs) to facilitate binding to neutrophils *in vivo*. Brain-targeted delivery to the basolateral amygdala (BLA) was demonstrated by enhanced concentration of baicalein in the BLA. An enhanced anti-depressant effect was observed *in vitro* and *in vivo*. The mechanism involved inhibition of apoptosis and a decrease in lactate dehydrogenase release. Behavioral evaluation carried out with rats demonstrated that anti-depression outcomes were achieved. The results indicate that PGP-SLNs decrease immobility time, increase swimming time and

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climbing time and attenuate locomotion in olfactory-bulbectomized (OB) rats. In conclusion, PGP modification is a strategy for targeting the brain with a cell–nanoparticle delivery system for depression therapy.

1. Introduction

Depression is one of the most common mental disorders in the world and is frequently observed as a comorbidity in clinical settings[1]. About 350 million people currently suffer from depression. The World Health Organization has predicted that, by 2020, depression will be one of the two top causes of global health burden and disability[2]. The health effects of depression are due to its contribution to mental disorders such as schizophrenia, bipolar disorder, schizoaffective disorder and drug addiction. Despite extensive studies, depression's etiology, pathogenesis, diagnosis and treatment has not been fully elucidated. In fact, depression covers a broad spectrum of disorders, which are multifactorial in origin including genetic, developmental, and environmental factors[3,4]. On one hand, it is believed that depression is associated with the alteration of neurotransmitter expression with structural changes within the brain and in the hypothalamus–pituitary–adrenal (HPA) axis; on the other hand, there is growing evidence indicating that inflammation accompanied by increased oxidative and nitrosative stress may play a crucial role in the pathogenesis of depression[5,6].

Accumulated evidence has shown that an inflammatory process in the brain is found in several psychiatric diseases including depression[7]. Leukocytes recruitment means monocytes and neutrophils can cross the blood–brain barrier, a major barrier for brain disease therapy. Phagocytosis and the unique extravasation property of leukocytes make it possible to exploit these cells as a carrier system for targeted drug delivery[8]. In fact, we have use monocytes as drug carriers for many brain disease therapies including depression[9–11]. *N*-Acetyl Pro–Gly–Pro (PGP) exhibits high binding affinity and specificity to neutrophils through the CXCR2 receptor[12,13]. In our previous studies PGP was used as a ligand to modify nanoparticles for binding with neutrophils *in vivo*, which are known to accumulate in lesions in ischemic stroke. Hence, it may be possible to design solid lipid nanoparticles modified with PGP peptide (PGP-SLNs) for brain-targeted delivery through neutrophils for depression therapy.

Baicalein (BA, 5,6,7,-trihydroxyflavone), one of the most active natural plant flavonoids, is found in the dry roots of *Scutellaria baicalensis* Georgi. It exhibits several beneficial actions, including effects in the central nervous and immune systems[14,15]. It has been widely used for the treatment of inflammation, hypertension, cardiovascular disease and bacterial infection, with the mechanism related to anti-neuroinflammation. It is reported that BA treatment improved motor impairments, attenuated brain damage, suppressed proinflammatory cytokines, modulated astrocyte and microglia activation, and blocked the activation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) signals in rotenone-induced rats[16].

Solid lipid nanoparticles (SLNs) are nanospheres made from solid lipids with a diameter of approximately 50–1000 nm. A solid lipid matrix, including glycerides, fatty acids or waxes, and stabilized by physiologically-compatible emulsifiers such as phospholipids, bile salts, Tween 80, polyoxyethylene ethers, or polyvinyl alcohol were often used for SLNs due to their low toxicity. The most common strategy for nanocarrier targeting to the brain is receptor-mediated endo-/transcytosis and cell-mediated delivery. This pathway depends on interaction of the surface ligand nano-carriers (transferrin, transferrin-receptor binding antibody, lactoferrin, melanotransferrin, folic acid and a-mannose or cRGD) with specific receptors, formation of endocytotic-vesicles that envelop the nanocarriers, transcytosis across the BBB, and exocytosis of the nano-carriers in the CNS parenchyma[17,18].

Hence, PGP peptide was used as a ligand for neutrophils as reported previously[19], allowing brain drug delivery of nanoparticles loaded with BA. The targeting efficiency, anti-oxidative mechanism, and anti-depressant effects in model animal were evaluated. An enhanced therapeutic outcome of PGP-SLNs loaded with BA (PGP-BA-SLNs) was observed in this study.

2. Materials and methods

2.1. Materials

Glyceryl monostearate (GM) and poloxamer 188 were obtained from China National Pharmaceutical Group Corporation (Beijing, China); BA was purchased from Meilunbio (Dalian, China); coumarin 6 (C6) and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI) were from FanboBio chemicals (Beijing, China); 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was obtained from Merck (Schaffhausen, Switzerland); PGP-PEG-DSPE and T7-PEG-DSPE were synthesized in our laboratory as previously reported[19,20]. A cell counting kit (CCK-8) was obtained from Sigma–Aldrich (Schnelldorf, Germany); the lactate dehydrogenase (LDH) release assay kit was obtained from Beyotime (Nantong, China); ultrafiltration tubes were from Millipore (3 kDa, Bedford, MA, USA); Hank's balanced salt solution (HBSS) was obtained from Invitrogen (Carlsbad, CA, USA).

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 Ethics Committee of The Second Hospital Affiliated Heilongjiang University of Traditional Chinese Medicine (Harbin, China; SYXK-2013-012).

2.2. Cell culture

The PC12 cell line (a neuron-like rat pheochromocytoma cell line) and the HL-60 cell line (the human promyelocytic leukemia cell line) were purchased from the Cell Bank at the Chinese Academy of Sciences (Shanghai, China). HL-60 cells were cultured in Iscove's

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