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Development, Characterization and Evaluation of Lactoferrin Conjugated and Memantine Loaded Peg-Plga Nanoparticles for the Treatment of Alzheimer's Disease

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Alzheimer's disease is a degenerative neurological condition that has no cure and only a few treatment options. It has a negative impact on one's cognitive and behavioural abilities. Traditional medications, such as acetylcholinesterase inhibitors, are often ineffective because they do not penetrate the blood-brain barrier. Targeted therapy strategies involving nanoparticulate drug delivery devices have been employed to improve the efficacy of Alzheimer's disease treatment. Memantine is an Alzheimer's disease medicine that has been approved for the treatment of mild to moderate symptoms. To boost memantine's action at the target region, we employed a twofold emulsion technique to generate lactoferrin (Lf) combined with biodegradable PEG-PLGA nanoparticles (NPs). The average particle size of the synthesised NPs was 162.60.5 nm, with a polydispersity index of 0.1 and a surface charge of -21.5 mV. The crystalline drug was disseminated within the PLGA matrix, according to the physicochemical characterisation of NPs. During in vitro dissolution studies, the new nanoparticulate formulation displayed a sustained release profile of memantine. The NPs were noncytotoxic to brain cell lines, and bEnd.3 cells had a greater concentration of Lf-NP than unconjugated nanoparticles. The researchers discovered that Lf-conjugated PEG-PLGA nanoparticles carrying memantine are excellent for Alzheimer's disease targeted delivery.

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Keywords: Alzheimer's disease; bEnd.3; lactoferrin; memantine; PLGA nanoparticles; PEG.

1. INTRODUCTION

Alzheimer's disease (AD) is the leading cause of dementia in the elderly [1]. Patients, family, and society are all affected by this condition, which is marked by a gradual deterioration of cognitive and non-cognitive functions. This chronic and progressive neurological disorder involves a large number of neurotransmitters, and the proportional contribution of each neurotransmitter to clinical symptoms is not fully known. AD and other neurodegenerative illnesses are difficult to treat due to the blood-brain barrier (BBB) [2]. The BBB's specific permeability is the most significant barrier to treating AD [1]. There are currently just a few medications available to treat AD due to the BBB's limitation on drug diffusion across the brain. While the BBB prevents hazardous xenobiotics from entering the CNS. It also prevents neuroprotective drugs from entering the central nervous system. To get around the BBB, either the drug's physicochemical qualities need to be changed to make it lipid-soluble, or the drug's size needs to be reduced to a tiny scale [3-4].

The current Alzheimer's disease (AD) treatment strategy focuses on vascular prevention and symptomatic treatment using cholinesterase inhibitors and NMDA antagonists. Memantine is a glutamate receptor antagonist that is non-competitive [5]. Excessive activation of neuronal amino acid receptors results in glutamate-related excitotoxicity, which plays a role in Alzheimer's disease pathogenesis [6]. Memantine works by inhibiting NMDA receptors in the glutamatergic system, lowering glutamate activity in brain cells and reducing neurotransmitter function. The interaction of memantine with NMDA receptors is critical for the drug's therapeutic effectiveness in Alzheimer's disease. Consumption of memantine, on the other hand, might produce dizziness, disorientation, constipation, and vomiting [7].

Engineered nanoparticles (NPs) with new physicochemical characteristics and the capacity to traverse the BBB might be a potential technique for overcoming biological and pharmacological hurdles in Alzheimer's disease treatment [8]. The targeted delivery of medications is a fundamental benefit of nanoparticles in the treatment of Alzheimer's disease [9]. PEGylation of the NPs improves their retention by extending their circulation time [10]. Furthermore, ligand conjugation is a very efficient way to improve the targeting efficacy of NPs [11]. Lactoferrin (Lf) is a promising targeting molecule that has the potential to improve delivery to the brain. Lf receptors (LfR) are located on the BBB and are responsible for the transport of Lf across the BBB [12]. It is also reported that Lf has a substantially higher brain uptake than transferrin and OX26 [13]. Hence the objective of the present study was development and characterization of Lf conjugated PEG-PLGA nanoparticles loaded with memantine for the treatment of Alzheimer's disease.

2. METHODS

2.1 Materials

PEG-PLGA polymer and Lactoferrin was obtained from Sigma-Aldrich and memantine (MEM) was procured from Dellwich Healthcare LLP, Ahmedabad. All tests were conducted with water filtered through the Millipore MilliQ system, and all other reagents were of analytical quality.

2.2 Preparation of Memantine Loaded PEG-PLGA Nanoparticles

The organic phase was formed by dissolving 50 mg of PLGA–PEG in 5 mL of ethyl acetate [14]. MEM was dissolved in deionized water to make the aqueous phase. To generate the main emulsion, the aqueous phase was introduced to the organic phase at a steady flow rate under severe shear using a probe sonicator [15]. To stabilise the colloidal system, the resulting mixture was dispersed in 2 ml of deionized water containing PVA (0.3 percent) and agitated for 2 hours with a magnetic stirrer [16-17]. The organic solvent was then evaporated using a rotavapor (Steroglass, Italy) under vacuum, and the NPs were washed by centrifugation at 15,000 rpm for 20 minutes. The identical process was used to load rhodamine into NPs [18].

2.3 Preparation of Memantine loaded Lf-PEG-PLGA Nanoparticles

To prepare the Lf-PEG-PLGA NPs, purified thiolated Lf was added to the PEG-PLGA NPs and incubated at room temperature for 9 hours. After passing the solution through a 1.5 cm x 20 cm sepharose CL-4B column, it was eluted with 0.01 M phosphate buffered saline (PBS) buffer

pH 7.4 to remove the unconjugated thiolated Lf [19].

2.4 Particle Size, Morphology and Zeta Potential

A laser diffraction particle size analyzer was used to determine the size of the nanoparticles (Cilas 1604L, France). The size of the vesicles was evaluated by suspending prepared nanoparticles in a particle size analyzer chamber containing milli-Q water [20]. NP zeta potential and polydispersity index (PI) were measured using photon correlation spectroscopy (PCS) using a ZetaSizer Nano ZS (Malvern Instruments) [17]. The nanoparticles were morphologically examined using a transmission electron microscope (TEM, Morgani 268D, Holland) after staining with a 1 percent (w/v) phosphotungstic acid solution [21-22].

2.5 Encapsulation Efficiency

The amount of drug encapsulated in nanoparticles was determined indirectly. Previously to the analysis, the non-loaded drug was separated from NPs by centrifugation at 14,000 rpm and filtered through 500Da MWCO. The encapsulation efficiency (EE) was calculated by the difference between the total amount of drug and the free drug, present in the filtered fraction [23].

2.6 *In vitro* Drug Release

In vitro drug release of MEM from PEG-PLGA NPs and Lf conjugated PEG-PLGA Nps was studied against free MEM in phosphate-buffered saline (PBS) [17]. Briefly, a volume of 5 ml of each formulation was placed directly into a dialysis bag (cellulose membrane, 500 Da Himedia, Mumbai) and each bag was placed on 100 ml of PBS pH 7.4 at 37°C. 1 ml of sample was removed from the stirred release medium at predefined intervals and replaced with 1 ml of new buffer at the same temperature. HPLC was used to determine the amount of drug released at each time point [22].

2.7 *In vitro* Cellular Uptake of Drug-loaded PEG-PLGA NPs and Lf Conjugated PEG-PLGA NPs

2.7.1 Cell culture

Dulbecco's Modified Eagle Medium containing 10% FBS, penicillin (100 U/ml) and streptomycin (100 mg/ml) was used to culture the immortalized mouse brain endothelial cell line b.End3 in 10 cm tissue culture dishes.

2.8 Cellular Uptake and Competition Assay of MEM loAded Lf Conjugated PEG-PLGA NPs and PEG-PLGA NPs

bEnd 3 cells were seeded at a density of 105 cells/cm2 onto 24-well plates. A suspension of nanoparticles (1–60g/ml) was added to the pre-incubated cells with HBSS for 15 minutes on the second day and incubated for 1 hour at 37^0C. The analysis for done for uptake of nanopartuculate formulations. For the competition assay, bEnd.3 cells were treated with Lf in RPMI solution (1 mM) and incubated for 30 min in advance. Afterwards, Lf treated and untreated cells were exposed to rhodamine loaded Lf conjugated PEG-PLGA NPs and maintained for 2 h at 37oC. The cells only with Lf solution were utilized as a blank control. Then the cells were washed three times with PBS (pH 7.4) subjected to observe with a Confocal Imaging microscope (FV3000, Olympus) [23,24].

3. RESULTS AND DISCUSSION

The double emulsion evaporation process was adopted for the fabrication of PLGA NPs because it is well suited for loading hydrophilic drugs such as MEM. The active Lf on the surface of the nanoparticles would ensure that the nanoparticles were targeted to the Lf receptor on brain capillaries. Knowing that the mean particle size is a key parameter for NPs to pass through the BBB, the purpose of this study was to produce NPs with a mean particle size of 100 and 200 nm. The average particle size of drug loaded PEG-PLGA NPs was found to be around 120 nm with the zeta potentials of around −21.5 mV. After Lf conjugation the nanoparticle size raise to around 162.6±0.5 nm (Fig. 1). The polydispersity index for formulation was found to be < 0.1 showing a monomodal distribution. The size of the prepared NPs was all below 200 nm that was regarded as favorable to brain transport.

TEM images revealed that the Lf conjugate PEG-PLGA NPs had a spherical shape. The results of in vitro release investigation done at a temperature of 37^0C in PBS pH 7.4. Following the initial burst phase, the drug released slowly from the polymeric matrix into the release medium. The initial burst release of drug could be attributed to the unloaded MEM portion, which is only weakly bound to the surface of the NPs due to the PEG coating. In vitro drug release

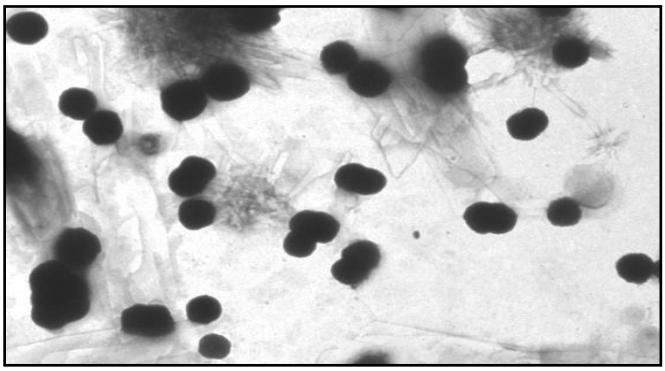

Fig. 1. TEM photograph of MEM loaded Lf conjugated PEG-PLGA NPs

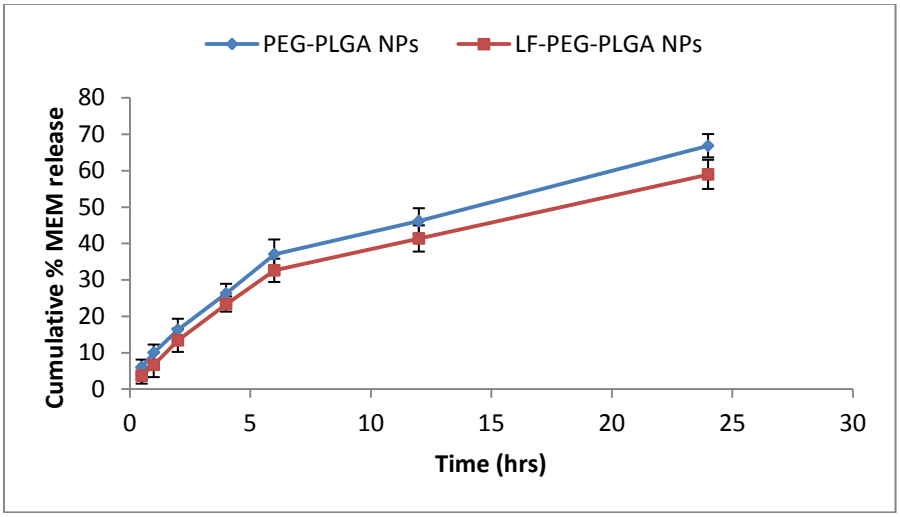

Fig. 2. *In vitro* drug release from prepared nanoparticles. ±SD (n=3)

study also shown that around 66.82% of MEM was releases in 24 hrs from MEM-loaded PEG-PLGA NPs and 58.95% of MEM was releases in 24 hrs from Lf conjugated PEG-PLGA NPs, confirming the slower release of the drug from the prepared nanoformulation (Fig. 2).

The cellular uptake of prepared nanoparticles in bEnd.3 cells were investigated to evaluate the targeting potential of the formulation. As a model for the BBB, bEnd.3 cells are a good choice because of their rapid growth, capacity to maintain blood-brain barrier properties through repeated transit, creation of functional barriers, and openness to a wide range of molecular treatments. A concentration-dependent in vitro uptake result for rhodamine-loaded Lf-NP by bEnd.3 cells indicated an endocytosis process. The uptake of Lf conjugate PEG-PLGA NPs by bEnd.3 cells was higher than the uptake of PEG-PLGA NPs. The uptake of Lf conjugate PEG-PLGA NPs increased with increase in the concentration.

In the competition assay, the cellular uptake of Lf conjugate PEG-PLGA NPs formulation was significantly higher than PEG-PLGA NPs formulation. After presaturation with free Lf, the fluorescence intensity of cells incubated with Lf conjugate PEG-PLGA NPs formulation was reduced, indicating that the decreased cellular uptake of Lf conjugate PEG-PLGA NPs formulation was due to free Lf binding competitively to receptors on bEnd.3 cells, further confirming Lf targeting effect on bEnd.3 cells via receptor mediated endocytosis.

4. CONCLUSION

Developing drug carriers with a wide range of features is now possible because of advancements in nanotechnology. These nanosystems could be used to deliver medicines and other neuroprotective drugs to the brain more effectively in treating Alzheimer's disease. In this study, a novel surface engineered brain drug delivery system was developed with an

average size lower than 200 nm and PI < 0.1, characteristic of mono dispersed systems, suitable to release the drug across the BBB. Developed formulation shown a sustained release profile of memantine. The significantly increased uptake of the Lf conjugate PEG-PLGA NPs by bEnd.3 cells compared with that of plain PEG-PLGA NPs was confirming the brain targeting potential of the developed carrier. In summary, MEM loaded Lf conjugate PEG-PLGA NPs could be a promising alternative towards a better treatment of AD patients since NPs have demonstrated to be capable to provide a more effective treatment than free MEM.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nagakura A, Shitaka Y, Yarimizu J, et al,. Characterization of cognitive deficits in a transgenic mouse model of Alzheimer's disease and effects of donepezil and memantine. Eur J Pharmacol. 2013;703(1–3):53–61.
2. Gothwal A, Kumar H, Nakhate KT, et al,. Lactoferrin coupled lower generation PAMAM dendrimers for brain targeted delivery of memantine in aluminum-chloride-induced Alzheimer's disease in mice. Bioconjug Chem. 2019;30(10):2573–83.
3. Pinheiro RGR, Coutinho AJ, Pinheiro M, et al,. Nanoparticles for targeted brain drug delivery: What do we know? Int J Mol Sci. 2021;22(21).
4. Parashar AK, Nema RK,. A Review on novel techniques for drug delivery to the brain. Current Research in Pharmaceutical Sciences. 2012;3:134-141.
5. Nakamura Y, Kitamura S, Homma A, et al. Efficacy and safety of memantine in patients with moderate-to-severe Alzheimer's disease: results of a pooled analysis of two randomized, double-blind, placebo-controlled trials in Japan. Expert Opin Pharmacother. 2014;15(7):913–25.
6. Matsunaga S, Kishi T, Iwata N,. Memantine monotherapy for Alzheimer's disease: A systematic review and meta-analysis. PLoS One. 2015; 10(4):e0123289.
7. Kurz A, Grimmer T,. Efficacy of memantine hydrochloride once-daily in Alzheimer's disease. Expert Opin Pharmacother. 2014;15(13):1955–60.
8. Cacciatore I, Ciulla M, Fornasari E, et al. Solid lipid nanoparticles as a drug delivery system for the treatment of neurodegenerative diseases. Expert Opin Drug Deliv. 2016;13(8):1121–31.
9. Cai Q, Wang L, Deng G, et al. Systemic delivery to central nervous system by engineered PLGA nanoparticles. Am J Transl Res. 2016;8(2):749–64.
10. Calvo P, Gouritin B, Chacun H, et al. Long-circulating PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery. Pharm Res. 2016;18(8):1157–66.
11. Jose S, Sowmya S, Cinu TA, et al,. Surface modified PLGA nanoparticles for brain targeting of Bacoside-A. Eur J Pharm Sci. 2014;63:29–35.
12. Huang R, Ke W, Liu Y, et al. Gene therapy using lactoferrin-modified nanoparticles in a rotenone-induced chronic Parkinson model. J Neurol Sci. 2010;290(1–2):123–30.
13. Moos T, Morgan EH,. Restricted transport of anti-transferrin receptor antibody (OX26) through the blood-brain barrier in the rat:

OX26 transport into brain. J Neurochem. 2001;79(1):119–29.
14. Meng FT, Ma GH, Qiu W, et al,. W/O/W double emulsion technique using ethyl acetate as organic solvent: effects of its diffusion rate on the characteristics of microparticles. J Control Release. 2003; 91(3):407–16.
15. Sanchez-Lopez E, Ettcheto M, Egea MA, et al. New potential strategies for Alzheimer's disease prevention: pegylated biodegradable dexibuprofen nanospheres administration to APPswe/PS1dE9. Nanomed Nanotechnol Biol Med. 201713:1171–82.
16. Cruz LJ, Stammes MA, Que I, et al, 2016. Effect of PLGA NP size on efficiency to target traumatic brain injury. J Control Release. 2016;223:31–41.
17. Sanchez-Lopez E, Ettcheto M, Egea MA, et al. Memantine loaded PLGA PEGylated nanoparticles for Alzheimer's disease: in vitro and in vivo characterization. J Nanobiotechnology. 2018;16(1).
18. Abrego G, Alvarado HL, Egea MA, et al. Design of nanosuspensions and freeze-dried PLGA nanoparticles as a novel approach for ophthalmic delivery of pranoprofen. J Pharm Sci. 2014; 103(10):3153–64.
19. Hu K, Shi Y, Jiang W, et al. Lactoferrin conjugated PEG-PLGA nanoparticles for brain delivery: preparation, characterization and efficacy in Parkinson's disease. Int J Pharm. 2014; 415(1–2):273–83.
20. Parra A, Mallandrich M, Clares B, et al. Design and elaboration of freeze-dried PLGA nanoparticles for the transcorneal permeation of carprofen: Ocular anti-inflammatory applications. Colloids Surf B Biointerfaces.2015;136:935–43.
21. Zhang C, Wan X, Zheng X, et al, 2014. Dual-functional nanoparticles targeting amyloid plaques in the brains of Alzheimer's disease mice. Biomaterials. 2015;35(1):456–65.
22. Parashar AK, Singh G. Synthesis and characterization of temozolomide loaded theranostic quantum dots for the treatment of brain glioma. Jou. of Med. P'ceutical & Allied Sci. 2021;10-I 3, 1073, 2778–2783.
23. Parashar AK, Singh G,. Synthesis and characterization of ligand anchored poly propyl eneimine dendrimers for the treatment of brain glioma. Jou. of Med. P'ceutical & Allied Sci. 2021;10-I 3, 1084, 2784–2789.
24. Pedros I, Petrov D, Allgaier M, et al, Early alterations in energy metabolism in the hippocampus of APPswe/PS1dE9 mouse model of Alzheimer's disease. Biochim Biophys Acta. 2014;1842(9):1556–66.

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