Formulation development and evaluation of nanoparticles for sustained delivery of levodopa-dendrimer conjugate for treatment of Parkinson’s disease

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ABSTRACT

Fluctuating levels of dopamine in brain is one of the major reasons behind development of Alzheimers and Parkinsons disease (PD). Alpha synuclein (AS) is a protein whose aggregation is believed to be the main reason behind aforementioned diseases. Levodopa is most common drug used for treatment of PD, but has many side effects due to fluctuating levels in blood. Delivering levodopa at a constant rate has thus gathered a lot of attention as a treatment strategy for Parkinson’s disease since it also inhibits the aggregation mechanism of AS. Here we demonstrate a method to synthesize levodopa-dendrimer conjugate (LDC) which will be further encapsulated in polymeric nanoparticles. We further investigate the release pattern from nanoparticles and its ability to inhibit AS aggregation.

INTRODUCTION

Recent reports have proven that alpha synuclein fibrillation leads to formation of lewy bodies which play an important role in inducing parkinson’s like symptoms.¹ Therefore, a drug which can inhibit the rate of fibrillation of alpha synuclein is expected to potentially reduce the disease symptoms. Levodopa is the gold standard for treatment of PD. However, its dose needs to be increased with chronic use and there are some side effects like nausea and vomiting associated with the metabolic products of levodopa such as 3-orthomethyldopa and dopamine. Fluctuating levels of dopamine causes wearing off effect. The best way to solve these problems is to deliver a sustained amount of levodopa to the brain so that a constant level of dopamine is maintained at the dopamine receptor sites.²

EXPERIMENTAL METHODS

Levodopa (1mg/ml) and PAMAM dendrimer (1mg/ml) were conjugated by mixing in deionized water at alkaline pH 8.5 (0.1N NaOH) and then freeze dried to remove solvent. The levodopa-dendrimer conjugate (LDC) was characterized using FTIR, DSC and UV. This conjugate was used to prepare nanoparticles (NP). Nanoparticles were prepared by forming a primary emulsion between glyceryl monooleate and water with Tween80 as the emulsifying agent. Cross-linked chitosan was added to form an extra layer of polymer in order to further control the release of LDC. The nanoparticles were characterized for particle size, zeta potential, release profile, drug loading efficiency and were investigated for their activity against fibrillation of alpha synuclein (AS) - a pathophysiological marker of Parkinson’s disease. Release of LDC was further investigated to understand the mechanism of release. AS-fibrillation was induced by incubating it (1mg/ml in 20 µM tris buffer pH 7.4) at 37°C with constant stirring at 600 rpm. The extent of AS fibrillation was evaluated quantitatively by using Thioflavin t assay (excitation and emission at 440 and 490 nm, respectively) and FTIR and qualitatively by light microscopy. Alpha synuclein fibrillation was evaluated in presence of equimolar solution of levodopa, levodopa-dendrimer conjugate, and nanoparticles containing conjugate

RESULTS AND DISCUSSION

LDC formation was confirmed by FTIR (Figure 1). The loading efficiency for nanoparticles containing 15% w/w LDC is 100%. The particle size and charge on nanoparticles was found to be in the range 236.6±6.9 nm and -24.6±2.8mv respectively. The nanoparticles showed a burst release of about 50%, but sustained the release of drug for further 4 days (Figure 2). The release profile of LDC was further plotted using the Higuchi model of drug release (Figure 3). Linear relationship between the cumulative release and square root of time proved that the release occurred by diffusion mechanism.

Fig 1. Formation of amide bond between levodopa and dendrimer confirmed using FTIR.
Levodopa solution alone fails to inhibit the fibrillation till 2nd day but manages to disrupt the preformed fibrils and inhibit further fibril formation till 10th day. However, no fibrillation was observed with nanoparticles. The length of the fibrils measured using light microscopy increased with time in control solution from 500 nm to 5 µm, which were not detected in the presence of either conjugate alone or nanoparticles containing conjugate solution (Figure 4).

CONCLUSION

Conjugation between levodopa and dendrimer was confirmed using DSC, FTIR and UV absorbance. The conjugate was able to inhibit AS fibrillation at a significantly higher rate than levodopa alone. The sustained release of LDC will help maintain a constant level of dopamine thereby leading to fewer side effects and better control over Parkinson’s like symptoms.

REFERENCES


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