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Thermosensitive PLA based nanodispersion for targeting brain tumor via intranasal route

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ABSTRACT

Delivery of drugs to the brain via nasal route has been studied by many researchers. However, low residence time, mucociliary clearance and enzymatically active environment of nasal cavity pose many challenges to successful nasal delivery of drugs. We aim to deliver methotrexate by designing thermosensitive nanodispersion exhibiting enhanced residence time in nasal cavity and bypassing the blood brain barrier (BBB).

PLA nanoparticles were developed using solvent evaporation technique. The developed nanoparticles were further dispersed in prepared thermosensitive vehicle of poloxamer 188 and Carbopol 934 to impart the property of increased residence time. The formulated nanoparticles demonstrated no interaction with the simulated nasal fluids (SNF), mucin, serum proteins and erythrocytes which demonstrate the safety of developed formulation for nasal administration. The penetration property of nanoparticles though the nasal mucosa was higher than the pure drug due to low mucociliary clearance. The developed nanoparticles diffused though the membrane pores and rapidly distributed into the brain portions compared to the pure drug. There was detectable and quantifiable amount of drug seen in the brain as demonstrated by in vivo brain distribution studies with considerably low amount of drug deposition in the lungs. The pharmacokinetic parameters demonstrated the enhancement in circulation half life, area under curve (AUC) and Cmax of the drug when administered intranasal in encapsulated form.

Thus, the thermosensitive nanodispersions are surely promising delivery systems for delivering anticancer agents though the nasal route for potential treatment of brain tumors.

1. Introduction

Glioblastoma multiforme is the most grievous type of brain tumors that remains poorly prognosed despite progress in chemotherapy and radiation [1]. The current therapeutic modalities used for the treatment of brain tumor include intracerebral administration, intraventricular injection and convection enhanced delivery involving disruption of the BBB [2,3]. The BBB is composed of endothelial cells limiting the passage of exogenous material into the brain and intravenous administration suffers a drawback in view of same [4]. Researchers have made several attempts to design delivery system that bypasses or circumvents this barrier thus allowing passage of actives into the brain. One of the strategies that had been tried for brain delivery of drugs is intranasal delivery since it bypasses the BBB. Intranasal delivery provides a practical, noninvasive method for delivering therapeutic agents to the brain because of the unique anatomic connections between the two by the olfactory and trigeminal nerves. Intranasally administered drugs reach the brain parenchyma, spinal cord, and cerebrospinal fluid (CSF) within minutes by using an extracellular route viz. through perineural and/or perivascular channels along the olfactory and trigeminal nerves without binding to any receptor. The drug can also be transported into the brain following axonal transport. In addition to bypassing the BBB, advantages of intranasal delivery include rapid delivery to the CNS, avoidance of hepatic first-pass drug metabolism, reducing unwanted systemic side effects and elimination of the need for systemic delivery. Intranasal delivery also provides painless and convenient self administration for patients encouraging its use for delivering therapeutic agents into the CNS [5].

There are numerous reports on intranasal delivery of drugs for treatment of CNS disorders like Parkinsonism, Gliomas and Alzheimer's disease warranting the delivery of drug into the brain [6]. Drugs like Neurotrophic factors such as NGF, antibiotic cephalexin, AVP, CCK analog, MSH/ACTH, insulin, calcitonin, methotrexate, estrogen and

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progesterone [7–16] have been delivered by this route. Intranasal route has enabled sufficient passage of drugs into the brain that otherwise does not cross the BBB. However, the concentration of the drug achieved in the brain is still low due to the enzymatic environment of the nasal cavity, low pH of the nasal epithelium, possibility of mucosal irritation and patient to patient variability [17]. Protection of the loaded agent from degradation in nasal enzymatic milieu and better absorption through the membrane has been achieved by loading of drugs into nanoparticles. However, the intranasally administered nanoparticles can be easily cleared by the mucociliary movement (nasal cavity is cleared every 20 min) causing loss of the active agent [18]. This can probably be avoided by increasing the residence time of nanoparticles by using mucoadhesive agents. Thermosensitive mucoadhesive gels of sumatriptan against migraine had been successfully prepared and delivered by the nasal route in our lab earlier [19]. We hypothesized that encapsulation of the anti-cancer agent into bioadhesive (nano) particles would be a promising methodology for successful anti-cancer delivery to the brain through nasal route for treatment of brain tumor. Thus, the following research work was undertaken to fabricate nanoparticles loaded with anti-cancer agent in a thermosensitive base that would gel upon administration into the nasal cavity for the potential treatment of brain tumors. As per the current literature, such a strategy had not been tried to date for the delivery of drugs to the brain via nasal route. Poly–Lactic Acid (PLA) was selected as the polymer for fabrication of nanoparticles for this purpose. PLA is biodegradable, non-immunogenic, non-toxic and safe polymer approved by US-FDA for use [20]. Methotrexate is not used for treatment of gliomas due to its poor passage through the BBB (methotrexate being a hydrophilic drug). Thus, the main aim of the current work was to design mucoadhesive nanoparticles with increased residence tie, low mucociliary clearance and enhanced permeation through the nasal mucosa that would unload the drug into the brain by bypassing the BBB. In this purview, PLA nanoparticles loaded with methotrexate were prepared using solvent evaporation technique.

2. Materials and methods

PLA and Poloxamer 188 were obtained as a gift samples from Evonik Industries and BASF respectively. Methotrexate was a generous gift from Cipla Pvt. Ltd. Carbopol 934 was purchased from SD-Fine Pvt Ltd., Mumbai. Fluorescein isothiocyanate (FITC) and dialysis bags-50, (6–10 kDa) were purchased from Himedia Laboratories Pvt. Ltd. cell culture media, DMEM nutrient mix, fetal bovine serum (FBS), penicillin/streptomycin stock solutions and 0.25% Trypsin-EDTA were purchased from Invitrogen Co., USA. All the other chemicals were of analytical grades and used without further purification for the study.

2.1. Preparation of PLA nanoparticle incorporated into thermosensitive gels

2.1.1. Preparation of drug loaded nanoparticles

In the present investigation, two different type of PLA nanoparticles were prepared viz. methotrexate loaded nanoparticles (MTX-NP) and placebo nanoparticle (P-NP). Poly-Lactic Acid (PLA) nanoparticles loaded with methotrexate were prepared using solvent evaporation technique [21]. Briefly; PLA (75 mg) and methotrexate (20 mg) were solubilised in dichloromethane in a covered glass beaker. The polymer-drug organic solution was emulsified with 10 mL of 10% aqueous poloxamer 188 solution pre-chilled at 4 °C at 9500 rpm using Ultraturax IKA 18. The obtained emulsion was immediately poured into 10 mL of pre-chilled 5% aqueous poloxamer 188 solution kept on a magnetic stirrer. Organic solvent was allowed to evaporate from the emulsion leaving behind PLA nanoparticles. Process parameters such as stirring rate and stirring time were optimized for developing nanoparticles of desired particle size and entrapment efficiency. MTX-NP and P-NP were lyophilized to obtain dried form of the product for DSC, and other physicochemical characterization studies. Particles were lyophilized in a Martin Chist lyophiliser operated at primary drying temperature of −40 °C for 16 h and secondary drying temperature of 25 °C at a vacuum pressure of 0.01 mBar.

2.1.2. Preparation of thermosensitive gels

Carbopol 934 (0.1% w/w–0.5% w/w of the final formulation) was added to the PLA nanoparticle dispersion to prepare thermosensitive gels as described elsewhere with slight modification. In brief, accurately weighed amounts of Carbopol corresponding to concentrations of 0.1–0.5% w/w were sprinkled on the surface of water and dispersed by mixing with a magnetic stirrer. The dispersions were then homogenized for 10 min at 15,000 rev/min followed by sonication and kept at rest for 1 day before use. Muco-adhesiveness of the developed formulation was assessed using mucoadhesiveness testing apparatus with respect to the weight required to detach the nasal mucosa from the formulation was determined. The rheological properties of gel were assessed using a cone and plate Brookfield viscometer (model HBDV-I; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) at 25 °C.

2.2. Characterization of nanoparticles

2.2.1. Particle size, zeta potential, morphology, differential scanning calorimetry (DSC) and X-ray diffraction study (XRD)

Particle size and zeta potentials of MTX-NP and P-NP was carried out using a Zetasizer (Nano ZS 90, Malvern Instruments Ltd., Malvern, UK) equipped with a 4.0 mW internal laser, which works on the principle of dynamic light scattering. The samples were diluted with double-distilled water in a disposable polystyrene cell prior to the measurements to obtain a nanoparticle suspension with a concentration below 0.5 mg/mL (to avoid multiple scattering) [22]. All the measurements were performed at 25 °C, at a scattering angle of 90°. The intensity-weighed mean diameter of the bulk population of particles was given by the z-average diameter value of the particles. The zeta potential (ZP) was measured using a Zetasizer (Nano ZS 90, Malvern Instruments Ltd., Malvern, UK) and a folded capillary cell. The sample was diluted with double-distilled water in a disposable polystyrene cell prior to the measurements to obtain a nanoparticle suspension with a concentration below 0.5 mg/mL. The ZP measurement was carried out subsequently. All tests were conducted at 25 °C in triplicate. [22].

Morphological assessment of MTX-NP and PLA based nanodispersion was performed using transmission electron microscopy (TEM). Briefly, 50 μl of sample was taken on carbon film coated on copper grid and allowed to air dry after which it was treated with phosphotungstic acid for negative staining. The analysis was done 200 kV accelerating voltage with suitable magnification between 25× to 75,000×. [22]. Interaction between the drug and excipients (PLA, MTX, P-NP, MTX-NP) were determined using DSC using the earlier reported method. X-ray diffraction study was performed for MTX, PLA polymer, MTX-NP and P-NP using X ray diffractometer (Perkin Elmer). [23].

2.2.2. Determination of entrapment efficiency

High Speed centrifugation was performed to determine the entrapment efficiency (%) of the drug in the developed MTX-NP. Nanoparticles were separated from the suspension by centrifuging the nanoparticulate dispersion at 15,000 rpm on a REMI centrifuge and the supernatant was separated carefully. The resultant solution was suitably diluted using phosphate buffer pH 7.4. Absorbance of the diluted solution was recorded on a Jasco-UV Spectrophotometer at 303 nm and the amount of methotrexate present in the supernatant was estimated from the calibration curve. The percent entrapment was calculated from the following formulae:

$$\text{Entrapment Efficiency}(\%) = \frac{(\text{Amount of drug in MTX-NP}) - (\text{Drug in supernatant})}{\text{Total amount of drug taken initially}} * 100.$$