MANNOSE ANALOGUE DECORATED LIPIDIC NANOPARTICLES FOR TARGETED DRUG DELIVERY TO BRAIN GLIOMA

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Abstract

Chemotherapy for brain glioma has been of limited value due to the inability of transport of drug across the blood-brain barrier (BBB) and poor penetration of drug into the tumor. For overcoming these hurdles, surface conjugated lipidic nanoparticles were developed with novel mannose derivative for targeting brain glioma. Lipidic nanoparticle were prepared by solvent emulsification and evaporation process and consequently characterized by various techniques like Differential scanning calorimetery (DSC), Dynamic light scattering (DLS). Drug loaded lipidic nanoparticles were surface modified with mannose derivative using carbodiimide coupling. Conjugation was confirmed using Infrared spectroscopy (IR). Drug encapsulation and *in vitro* release studies were carried out using HPLC. Conjugated lipidic nanoparticles were found to give sustain drug release as compared to drug solution. The targeting effects were evaluated on the glioma cell lines (U-87 MG). Cell toxicity assay were performed and results were encouraging with remarkable decrease in IC50 values as compared to drug encapsulated unconjugated lipidic nanoparticles and drug control and was further corroborated with cell uptake assay. Drug loaded mannose derivative-conjugated lipidic nanoparticles showed better IC50 and improved cell uptake. Hence, these conjugated lipidic nanopartiles are efficient delivery vehicle to target drugs to brain tumors.

Keywords: Brain, glioma, blood brain barrier, lipidic nanoprticles, conjugation

INTRODUCTION

Glioblastoma multiforme (GBM) is the most frequent primary central nervous system tumor, which represents the second cause of cancer death in adults less than 35 years of age. Since GBM differs from other cancers by its diffuse invasion of the surrounding normal tissue, it is impossible to make the complete removal of tumor by the conventional surgical method and tumor recurrence from residual tumors is very possible. Consequently, it is critical to deliver the therapeutic agent effectively to the tumor as well as to infiltrating cells that are not located in the tumor bed for GBM treatment. However, the therapeutic effects of present chemotherapy are very limited, but often causing systemic side effects, because almost all large molecule drugs and more than 98% small molecule candidate drugs are unable to reach the brain tissue due to their poor permeability across the Blood-brain Barrier (BBB). Another obstacle in chemotherapy is maintaining a higher concentration of therapeutic agents at the tumor site and then preventing their spread into healthy tissue. To overcome the limitations of the conventional drug delivery methods, there is a need for a multifunctional carrier that can be engineered into a single nano-platform such that it can carry drugs cross the BBB and then target the tumor [1],[2],[3].

Improvement of drug uptake into brain is a tough job in the treatment of various brain disorders. We chosen our model drug as Docetaxel (DTX) because it is a drug with restricted brain uptake due to the BBB, which limits the penetration of DTX into brain. Lipidic systems are the efficient delivery systems to enhance the targeting potential. Compared to other nanoparticulate systems, Solid lipid nanoparticles, SLNs have more

advantages such as high drug loading capacity and simplicity to couple ligands to improve the targeting efficiency [4].

With this in mind, SLNs merit attention to serve as a versatile targeting platform due to their unique structural and functional surface groups that can be used for conjugating multifunctional ligands. SLNs can be designed to carry therapeutic agents that avoid interference with the immune system. Additionally, due to their small size, nanoparticles can easily flow through blood capillaries and enter the target cancer cells [5] [6] [7].

Recent facts have revealed that a family of structurally related facilitative stereospecific glucose carriers mediates the transport of glucose in brain and other tissues. Among the facilitative glucose transporters (GLUT), the GLUT1 isoform is mainly expressed in the luminal surface of the brain capillaries and the choroid plexus. Thus, GLUT1 could be potentially employed for enhancing the delivery of the carriers across the BBB. Like most of the nutrient transporters, GLUT1 mediates the transport of substances with similar structures of glucose across the BBB, including 2-deoxyglucose, galactose, mannose, and glucose analogue [8]. Furthermore, GLUT1 is also expressed by glioma cells in the brain [9], [10]. Taking into account the specific affinity to the GLUT1, a mannose analog was incorporated onto the surface of SLNs in our study.

MAIN TEXT (EXPERIMENTAL, RESULTS AND DISCUSSION)

Solid lipid nanoparticle preparation

Drug loaded SLNs were prepared by emulsification and solvent evaporation method using glyceryl monostearte, soy lecithin and stearic acid (SA) in lipid phase. Process and formulation variables such as homogenization time, sonication time, stirring time and surfactant concentration were optimized to get minimum particle size with narrow distribution and high drug loading efficiency.

Mannose analogue conjugation on SLN surface

Conjugation of Mannose analogue on surface of SLNs was done utilizing EDC/NHS coupling reaction between stearic acid of SLNs and mannose analogue. Conjugation was confirmed further using Infra Red (IR) spectroscopy

IR Spectroscopy

FT-IR spectrum amide peak confirmed the formation of amide bond between SA in the lipid phase and Mannose analogue.

Particle size, Zeta potential and Encapsulation efficiencies of SLN and Mannose anlogue conjugated SLN

DTX loaded SLNs prepared with 1.5 % Tween 80 showed minimum particle size of 78± 3.1nm with 98 ± 4.15% entrapment efficiency and good stability.

Further, particle size of surface conjugated SLNs was significantly increased (99±4.5 nm) and entrapment efficiency (85%) was decreased compared to SLNs due to the conjugation of mannose analogue on the surface of these SLNs.

However, the zeta potential of conjugated-SLNs was significantly decreased in comparison with other SLNs proving surface group modification.

Crystallinity studies

Crystallinity studies of these SLNs using Differential scanning colorimetry (DSC) indicated the successful inclusion of drug into the lipid. Decreased intensity or absence of drug peaks in the formulation demonstrated that the drug was amorphous or disordered crystalline phase after incorporating it into SLNs. The change in crystallinity of lipid and drug will influence the release of DTX from SLNs. DSC thermogram is shown in figure 1.

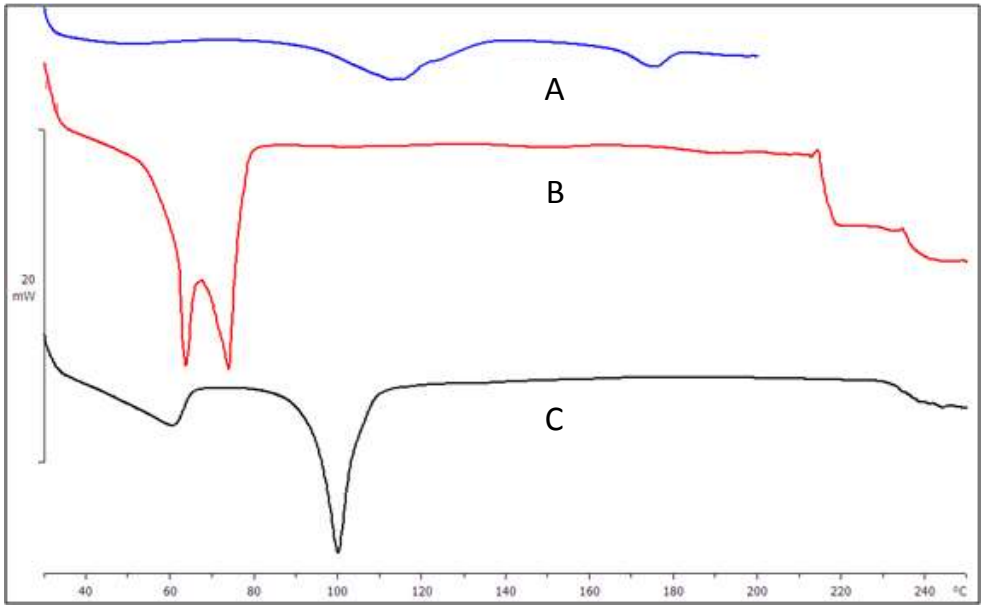

Figure 1: Differential scanning calorimetry (DSC) thermogram of A. DTX, B. PM+SLN and C. ADN-SLN.

***In vitro* release studies**

In vitro release studies were carried out using dialysis bag method. *In vitro* release of DTX from Conjugated SLNs showed controlled release of drug and the release was less compared with SLNs and drug solution (Figure 2). Because of surface modification with mannose analogue, particle size of C-SLNs was increased and that resulted in increased distance between the core and the surface of nanoparticles and hence the release of drug entrapped in mannose analogue conjugated SLNs was less at every time point compared to that from SLNs and drug solution.

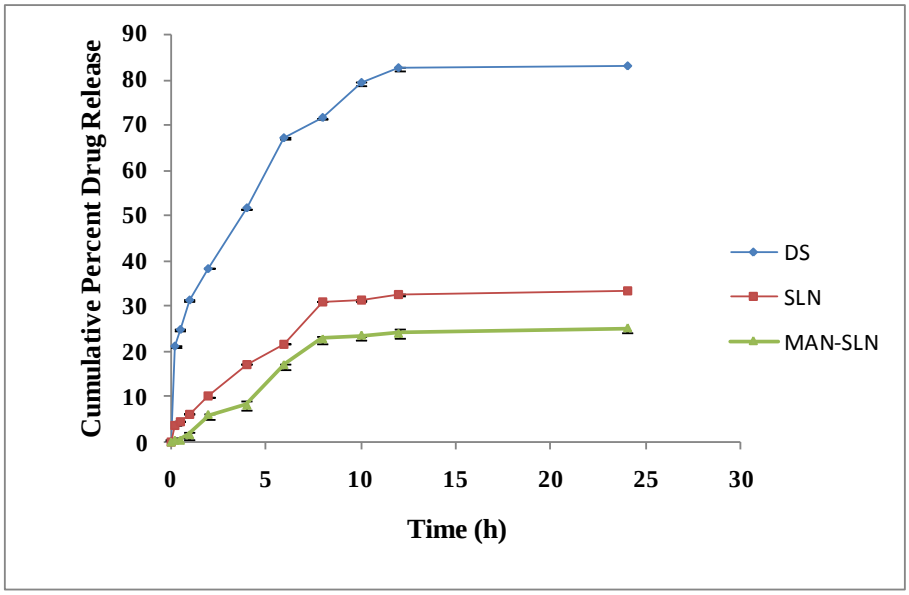

Figure 2: *In vitro* drug release studies of different DTX formulations presented as cumulative drug release vs time graph (mean ± SD; n = 3).

CYTOTOXICITY AND CELL UPTAKE STUDIES

Conjugated DTX loaded SLN showed significantly lower IC50 values when compared with either DTX solution or SLNs in U87 MG cells. These conjugated DTX loaded SLNs showed significant decrease in IC50 value compared with unconjugated DTX loaded SLNs after 24 h incubation in U87 MG cells. This is because of the increased uptake of DTX with conjugated SLNs compared with that of unconjugated SLNs. Therefore it is evident from the cell uptake study that surface modification of SLNs with this novel mannose analogue resulted in higher uptake of DTX from conjugated SLNs.

CONCLUSION

To conclude, a brain targeting drug delivery system was successfully constructed by carbodiimide-mediated coupling of SLNs with a newer mannose analogue, where it was used for active targeting to brain glioma. Mannose analogue–conjugated SLN was exhibited to be a potential active targeting drug delivery system for treatment of brain cancer as it possessed notable binding affinity and specificity towards brain cells, low cellular toxicity on normal and target cells, and better accumulation in brain tumor. Henceforward, this brain targeting delivery carrier will further be applied on animal model to estimate its antitumor efficacy.

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