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FORMULATE, OPTIMIZE AND CHARACTERIZE LACTOFERRIN CONJUGATED TEMOZOLOMIDE AND RESVERATROL CO-LOADED NLC FOR THE TREATMENT OF GLIOBLASTOMA

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Abstract

Among brain tumors, glioblastoma multiforme (GBM) is the most prevalent and potentially fatal kind. The location of the tumor, its heterogeneity, the presence of the blood-brain barrier (BBB), and other factors all contribute to the difficulty of treating GBM, and tumor recurrence is common even after treatment with surgery and chemotherapy. L/R-T/V-NLCs were shown to have a high efficiency in encapsulating drugs and a high stability at the nanoscale. While SLNs and NLCs serve a significant role as drug carriers in GBM treatment, we explore the key challenges in treating GBM in this study, and the full range of modification techniques that try to alter the SLN and NLC composition for the betterment of treatment results.

Key words: Gliomatosis cerebri, nanostructured lipid carriers, lactoferrin, arginine–glycine–aspartic acid peptide, vincristine, temozolomide

INTRODUCTION

Current therapy for GBM have limitations due to their inability to penetrate the blood-brain barrier (BBB), despite the fact that the prognosis is dismal. To overcome drug resistance, boost therapeutic efficacy, and minimize negative effects, combination chemotherapy is gaining popularity. 4 Brain tumors, on the other hand, have an urgent need for a unique targeted nanoparticulate delivery technology to increase solubility, lengthen circulation duration, boost targeted action, and finally lower systemic toxicity.

It takes very large dosages to reach therapeutic levels in the central nervous system due to their poor water solubility, difficulty to penetrate the blood-brain barrier, and lack of effectiveness, the vast majority of pharmaceuticals are associated with increased toxicity and unwanted side effects. To get over all these limitations, Researchers have developed nanodrug delivery systems that show promise as drug carriers for GBM therapy. SLNs are effective nanocarriers because they avoid the toxicity, limited loading capacity, and low stability that plagued previously studied nanocarriers. In addition, SLNs may be functionalized with a variety of ligands to transport therapies to the intended tissue. Unfortunately, SLNs tend to gel, and because there are no free sites for the medication to occupy during recrystallization, the drug is forced out. By fusing solid and liquid lipids to create an irregular crystal structure with more internal space, Müller et al. proposed NLCs as enhanced SLNs to increase entrapment efficiency during storage. The latest SLNs have improved stability and trapping efficiency. Nanostructured lipid carriers and solid lipid nanoparticles: insights into their role in GBM treatment, as well as new modification tactics in these systems, are provided in this study.

First identified at an iron-binding site, lactoferrin (Lf) is a double-lobe glycoprotein with the ability to reversibly chelate and transport iron. It belongs to the family of transferrins (Tf). Lf receptor expression was detected in GBM cells. As the conjugated Lf acts on receptor-mediated signaling pathways, it may be used to regulate transcytosis across the BBB and penetration to GBM.

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LITERATURE REVIEW

Hegde, M.M., Prabhu, S., Mutalik, S. *et al.* (2016), Despite significant advances in our knowledge of glioblastoma's (GBM) origins and spread, the illness still claims the lives of far too many people. Existing barriers, such as the blood-brain barrier, prevent current treatment techniques, which mostly consist of surgery followed by adjuvant chemoradiation, from adequately enhancing patients' chances of survival (BBB).

A. Chaudhuri; et al. (2018). Since no expressed receptors are present in triple-negative breast cancer, there are limited therapy options for this aggressive form of the disease in 2018. To combat TNBC, novel nanoparticles have emerged as a treatment option in an effort to boost the effectiveness of standard chemotherapies. Moreover, LNPs are able to circumvent physiological barriers, leading to a greater concentration of medicines at the intended site of action. Some liposomal formulations may one day be used in clinical practice if scientists put in sufficient effort; however, there are a number of obstacles that must first be overcome, including the formulations' relatively high cost, difficulties in scaling them up, and the need for more targeted delivery. In this study, we have assembled the current knowledge base on the many kinds of LNPs.

Garg, J., Pathania, K., Sah, S.P. et al., (2019) In this summary, the structure, categorization, components, and different preparation techniques shown by many research investigations are carefully outlined, along with the benefits and drawbacks of each. After introducing the idea of drug loading and release, we quickly covered stability and explored ways to make NLCs more stable. The present clinical state of NLCs has also been described to provide context for their potential contribution.

Van-An Duong, Thi-Thao-Linh Nguyen (2018), Solvent injection technique offers an alternate approach to creating SLNs and NLCs, which have been the subject of much research and study. Faster manufacturing, less difficult handling, and use in a wider variety of labs without the need for specialized equipment are just a few of the benefits of the solvent injection approach. Properties of SLNs and NLCs as a function of solvent injection technique parameters are also investigated.

Adryana Rocha Clementino and Fabio Sonvico (2018), One of the most intriguing uses of nasal delivery is getting medications into the brain without crossing the blood-brain barrier. The systemic adverse effects of powerful medications might be mitigated by measures such as reduced dosing and improved targeting using this method. Positive findings have been shown in recent clinical studies investigating the use of insulin delivered through nasal spray for the treatment of Alzheimer's disease. The development of nanomedicines offers new prospects for bringing nasal-to-cognitive transfer into clinical practice. In particular.

Preparation of L/R-T/V-NLCs

Figure 1 shows L/R-T/V-NLCs made using the solvent diffusion method. A lipid dispersion comprising 200 mg of SPC (10) and ATO (888) was prepared using Cremophor ELP (1 mL). After 24 hours of dialyzing L/R-T/V-NLCs against Milli-Q water, we introduced the lipid phase into the stirred aqueous phase. Following filtration via a 0.45 m pore size membrane and two washes in Milli-Q water, the L/R-T/V-NLCs solution was resuspended in PBS (pH 7.4) for long-term storage between 2°C and 8°C.

Without the addition of TMZ and VCR, Lf and RGD dual-ligand-comodified NLCs (L/R-NLCs) were generated in the same way.

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NLCs (L/R-T-NLCs) loaded with both Lf and RGD ligands and just one molecule of TMZ were produced in a similar fashion, but without the use of VCR.

NLCs (L/R-V-NLCs) loaded with both Lf and RGD were produced in the same way, but without the addition of TMZ.

The same procedure was used to manufacture L-T/V-NLCs with TMZ and VCR coloading but without RGD-PEG-DSPE.

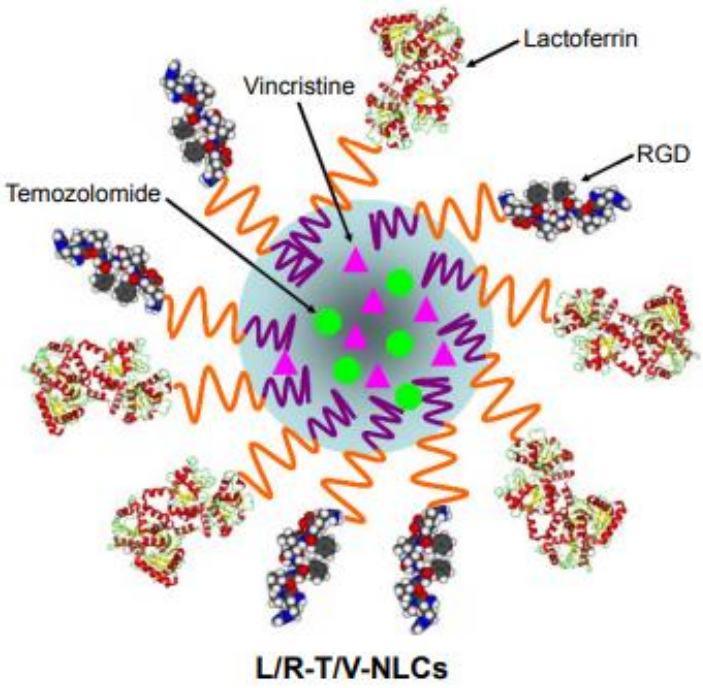

Figure 1 Scheme graph of L/R-T/V-NLCs.

Characterization of particle

Deionized water was used to dilute the suspensions to the appropriate concentration for the experiment. Size and charge on the NPs' surfaces were measured using a Nano-ZS Zetasizer DLS detector at 25 degrees Celsius. The polydispersity index described the extent to which the size distributions varied (PDI).

Efficiency in medication loading and encapsulation

To quantify the EE, we compared the overall quantity of TMZ to the amount that was encapsulated in the NLCs. The VCR was measured using HPLC. In conclusion, 20 L of the clear solution was treated with ethanol to disrupt the SLNs or NLCs and then introduced into an HPLC system. Acetonitrile and 0.01 M NaH2 PO4 were used as the mobile phase in conjunction with a Kromasil C18 reverse phase column to isolate the target substance. Mobile phase at 35 degrees Celsius was used to elute the samples, which were then examined at 297 nanometers (nm) in wavelength.

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Stability of NLCs

The techniques for calculating mean particle sizes and EE are detailed in the sections under "Characterization of Particle Size and Surface Charge" and "Characterization of Drug Encapsulation and Drug-loading Efficiency," respectively.

Results

Lf-PEG-DSPE's chemical structure was deduced using 1 H-NMR spectroscopy, and the corresponding structural changes have been labeled. Lf-PEG-DSPE production may depend on chemical changes in thioether bonding, amido linkages, Lf, PEG, and DSPE.

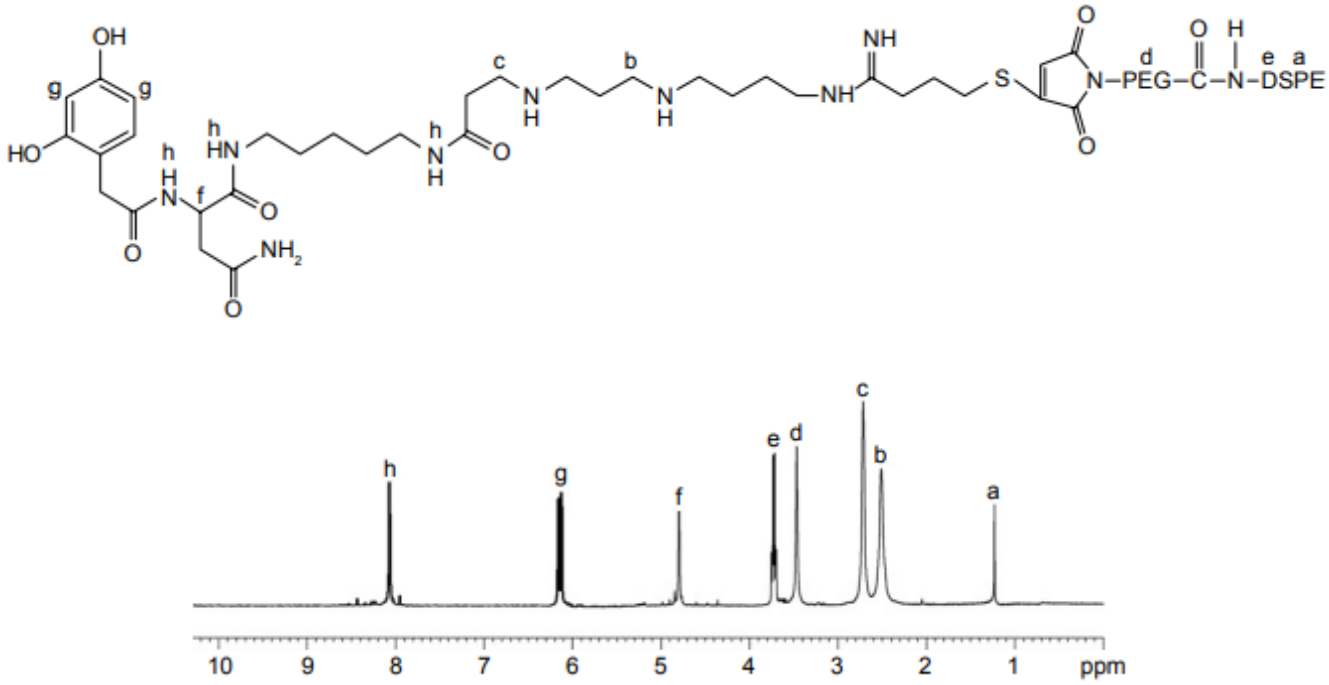

Figure 2: Lf-PEG-DSPE's molecular structure and 1 H-NMR spectroscop.

Preparation and characterization of NLCs

Compared to their ligand(s)-modified counterparts, the noligand-modified T/V-NLCs were much smaller, measuring in at just 96 nm. NLCs have positive zeta potentials, with the zeta potential of L/R-T/V-NLCs being +32 mV. In every single NLCs sample, the EE was above 80%. The DLs range from 5.3% to 10.1% throughout the different formulations. (Table 1).

Table 1 Characterization of different vectors

Formulations	Particle size (nm)	Size distribution (PDI)	Zeta potential (mV)	EE (%)		DL (%)	
				TMZ	VCR	TMZ	VCR
L/R-NLCs	133.9±3.2	0.135±0.019	31.8±2.1	N/A	N/A	N/A	N/A
L/R-T-NLCs	138.3±4.3	0.152±0.025	33.1±2.6	82.9±3.6	N/A	6.37±0.6	N/A
L/R-V-NLCs	139.7±4.1	0.166±0.028	30.9±3.0	N/A	81.4±3.7	N/A	5.5±0.4
T/V-NLCs	96.3±3.1	0.121±0.016	34.3±2.5	83.4±2.9	81.6±2.7	10.1±0.7	7.9±0.8
L-T/V-NLCs	135.5±3.8	0.163±0.023	27.6±2.9	83.1±2.8	80.8±3.3	7.8±0.6	6.1±0.5
R-T/V-NLCs	113.6±3.1	0.143±0.022	40.5±3.1	84.2±3.2	81.2±3.1	8.4±0.8	6.5±0.6
L/R-T/V-NLCs	139.3±4.9	0.187±0.021	32.4±2.7	81.9±3.4	82.2±3.2	6.7±0.7	5.3±0.5

NLC as Smart Drug Delivery Systems

During the last several years, many strategies for building a flexible nanoplatform have emerged. Without taking into account the numerous challenges associated to smart nanocarriers with optimum features, several methods of modifying nanoparticles for use as drug carriers in the body have been investigated. The term "smart drug delivery system" used to describe a vehicle that can transport a therapeutic or pharmaceutical to a specific set of cells while causing minimal harm to surrounding ones; this type of carrier is ideal for co-delivery of drug with another substance, such as genetic material, diagnostic agents, or even combined chemotherapy; it also has the added benefit of avoiding immune cleansing. Both SLNs and NLCs have been upgraded from their original forms to become "smart drug carriers" that can overcome all of the obstacles present in GBM treatment. (Figure 3).

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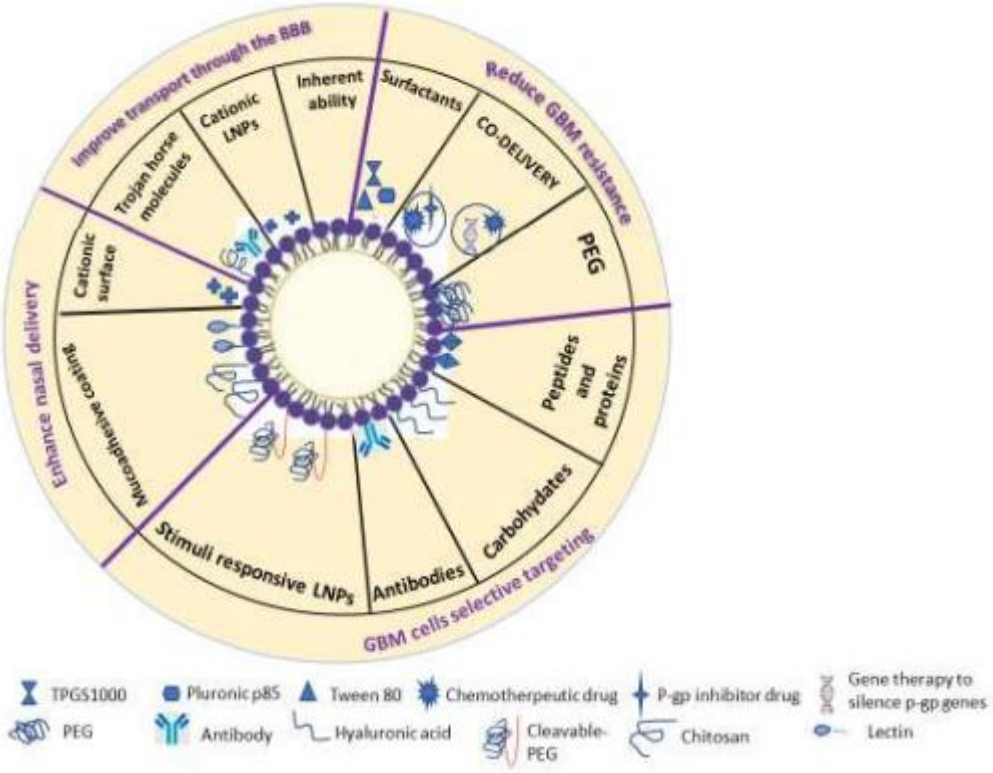

Figure 3. Improved therapy for glioblastoma multiforme with the use of nanostructured lipid carriers and solid lipid nanoparticles

Strategies for Improving Blood-Brain Barrier (BBB) Penetration in the Treatment of Glioblastoma Multiforme

The blood-brain barrier (BBB) is the primary biological barrier to successful GBM therapy. It is plausible that SLNs and NLCs, to a lesser degree, may penetrate the blood-brain barrier since they are lipids. Cationic nanoparticles in the AMT system and trojan-horse molecules on a nanocarrier in the RMT system are both often employed for this purpose. Angiopep-2 conjugated SLNs for docetaxel injection were shown to be more successful in killing cancer cells than unconjugated SLNs, according to research by Kadari A. et al. For the treatment of GBM, etoposide-loaded SLNs were conjugated with melanotransferrin antibody (MA) in another investigation. Tolerable toxicity to endothelial cells and enhanced transport and inhibitory action on GBM cells were seen using etoposide-loaded SLNs targeting the melanotransferrin antibody (MA-ETP-SLNs). There are a number of ways to create positively charged LNPs that may be employed with the AMT system to increase permeability across the BBB. Albumin, stearylamine, and protamine are all examples of cationized proteins that may be used in this way, as well as other cationic lipids and cell-penetrating peptides (CPP). In an effort to create cationic LNPs, 3beta-[N-(N',N'-dimethylaminoethane) carbamoyl] cholesterol was used to generate positively charged SLNs, which were subsequently conjugated to a monoclonal antibody against TfR (OX26).

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Table 2: SLNs and NLCs for enhanced treatment of GBM

Strategies to Enhance Crossing the BBB			
Formulation	**Cargo/drug**	**Ligand**	**Target**
SLN	Docetaxel	Angiopep-2	lipoprotein receptor related protein 1 (LRP1)
SLN	Etoposide	melanotransferrin antibody (MA)	Melanotransferrin
Cationic SLN	Biacalin	OX26 monoclonal antibody	Transferrin receptor (TfR)
Cationic SLN	Carmustine	Anti-EGFR	EGFR
SLN	Doxorubicin	Aprotinin, melanotransferrin antibody	low-density lipoprotein receptor (LDLR) related protein (LRP), melanotransferrin
SLN	Methotrexate	Bovine serum albumin (BSA)	Negative charge of BBB endothelial cells membrane

Modification Strategies Against GBM Cells Resistance			
Formulation	**Cargo/drug**	**Strategy**	**Target**
SLN	Edelfosine	Tween® 80	P-gp efflux
SLN	Trans-Resveratrol	TPGS	P-gp efflux
SLN	Noscapine	PEG	P-gp efflux
SLN	Curcumin, Piperine	TPGS and Brij 78	MDR effect
Folate SLN	Docetaxel	Ketoconazol	P-gp efflux
Strategies for Selective Targeting of GBM Cells			
NLC	Etoposide	Folic acid	Folate receptor
NLC	Etoposide	Folic acid, ρ-aminophenyl-α-D-manno-pyranoside (APMP)	Folate receptor, glucose transporter 1
SLN	Carmustine	Cetuximab	EGFR
NLC	Temozolomide	RGD peptide	Integrin receptors
SLN	Docetaxel	Lactoferrin	Lactoferrin receptors
SLN	Vorinostat	Hyaluronic acid	CD44
LNP	siRNA	PEGylated (poly (ethylene glycol)) cleavable lipopeptide	MMPs
Cationic SLN	camptothecin	Cleavable PEG	Tumor low pH
Modified Lipid Nanoparticles for Nose-to-Brain Delivery			
SLN	Pueraria flavone	Borneol	Improve crossing the BBB and permeability through nasal mucosa
NLC	Proteins	Chitosan	Prolonged interact with nasal mucosa

Perspectives on the Use of SLNs and NLCs in the Treatment of Glioblastoma

Because of the tumor's heterogeneity, resistance, aggressiveness, and invasive nature, as well as the difficulties of medication transport into the brain, GBM therapy is now regarded a severe challenge. The potential of SLNs and NLCs as drug carriers for treating GBM effectively is an exciting area of research that gives patients hope. This means that every method now used in the creation of SLNs and NLCs has to be critically examined. Our research suggests that additional time and energy should be spent developing the methods necessary to create nanocarriers at scale. Further research is needed to determine whether or not nanoparticles provide any health risks to organisms due to their ability to penetrate cell membranes and interact with different biological systems. To forecast the toxicity of nanoparticles in all organs, it could be useful to undertake in vivo testing to learn more about this. To

ensure the safety of nanoformulations designed for nasal-to-cerebral transfer, a chronic toxicity study is required in the lungs. Despite these setbacks, SLNs and NLCs have shown a great deal of potential as intelligent drug delivery systems for the treatment of malignant glioblastoma multiforme.

CONCLUSION

In this work, based on our findings, we offered L/R-T/V-NLCs as an option for treating GBM in conjunction with other conditions. The stability of the L/R-T/VNLCs was assured by their nanoscale size and high EE. Some of the advantages of L/R-T/V-NLCs were high cellular uptake, cytotoxicity, synergistic effects, improved drug accumulation in tumor tissue, and striking tumor suppression efficacy with minimal systemic toxicity. The use of L/R-T/V-NLCs as a drug delivery method for the treatment of glioblastoma has shown encouraging results.

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