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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

The tumor's location, its heterogeneity, and the existence of the blood-brain barrier (BBB) are all factors to consider, and other factors all contribute to the difficulty of treating GBM, and tumor recurrence is common even after treatment with surgery and chemotherapy. For the treatment of GBM, researchers here combined temozolomide and vincristine with nanostructured lipid carriers containing lactoferrin and arginine-glycine-aspartic acid dualligands. The primary difficulties in treating GBM are discussed, as is the crucial role that SLNs and NLCs play as drug carriers in GBM therapy, 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, temozolomide

INTRODUCTION

The median survival time for patients with GBM treated with resection, radiation, and temozolomide (TMZ) treatment was 14.6 months. Nonetheless, the poor prognosis may still be problematic; thus, there is an urgent need for techniques to breach the blood-brain barrier (BBB) and increase the effectiveness of existing GBM treatments. 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 an unique targeted nanoparticulate delivery technology to increase solubility, lengthen circulation duration, boost targeted action, and finally lower systemic toxicity.

To address these shortcomings, scientists have created nanodrug delivery methods, which have the potential to be the most efficient drug carriers for the treatment of GBM. SLNs are effective nanocarriers because they avoid the toxicity, limited loading capacity, and low stability that plagued previously studied nanocarriers such niosomes, transfersomes, micelles, liposomes, emulsions, and polymeric nanoparticles. 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. The latest SLNs have improved stability and trapping efficiency. This research sheds light on the role of nanostructured lipid carriers and solid lipid nanoparticles in GBM treatment and suggests some novel approaches to modifying these systems.

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. 14 As the conjugated Lf acts on receptor-mediated signaling pathways, it may be used to regulate transcytosis across the BBB and penetration to GBM. Malignant U87MG cells, a representative GBM cell line, have been demonstrated to have their proliferation inhibited by Lf owing to the down regulation of cyclins D1 and D4.

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. Increased loading capacity, greater temporal and thermal stability, lower therapeutic dosage and related toxicity, and decreased drug resistance are only a few of the therapeutic benefits of LNPs in comparison to traditional treatment and other nanoparticles. 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, their clinical status and toxicity, as well as the most recent developments reported for the treatment of TNBC in recent years.

J. Garg, K. Pathania, S.P. Sah, (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. Here, we take a look back at the recent developments of this technique for producing SLNs and NLCs. 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.

Preparation of L/R-T/V-NLCs

Figure 1 shows L/R-T/V-NLCs made using the solvent diffusion method. The lipid dispersion was made with Cremophor ELP and included 200 mg of SPC (10) and ATO (888). One milliliter of dimethyl formamide was added to 100 milligrams of injectable soy lecithin, 50 milligrams of TMZ, and 50 milligrams of VCR while the mixture was heated to 80 degrees Celsius to 85 degrees Celsius. Specifically, 10 mL of water was used to dissolve 100 mg of Lf-PEG-DSPE, 100 mg of RGD-PEG-DSPE, and 0.5% w/v DDAB to make the aqueous phase. After 24 hours of dialyzing L/R-T/V-NLCs against Milli-Q water, we introduced the lipid phase into the stirred aqueous phase.

- 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.

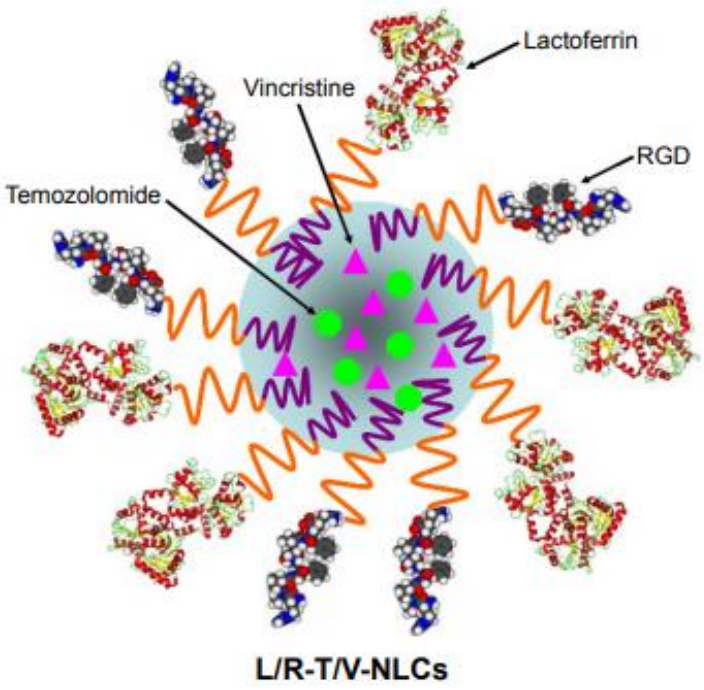

Figure 1 Scheme graph of L/R-T/V-NLCs.

To get the right concentration for the test, Deionized water was used to dilute the suspensions. 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).

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, After treating the clear solution with ethanol to disrupt the SLNs or NLCs, 20 L of the solution was put into an HPLC apparatus. Mobile phase at 35 degrees Celsius was used to elute the samples, which were then examined at 297 nanometers (nm) in wavelength.

Stability of NLCs

Over the course of 90 days, the size, PDI, and drug EE of NLC samples were assessed while the suspensions were held at 2°C-8°C. At the 0-, 10-, 20-, 30-, 60-, and 90-day marks, 22 data points are collected. 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

Structure confirmation of Lf-PEG-DSPE

Lf-PEG-DSPE's chemical structure was deduced using 1 H-NMR spectroscopy; structural modifications are denoted with letters. Lf-PEG-DSPE production may depend on chemical changes in thioether bonding, amido linkages, Lf, PEG, and DSPE.

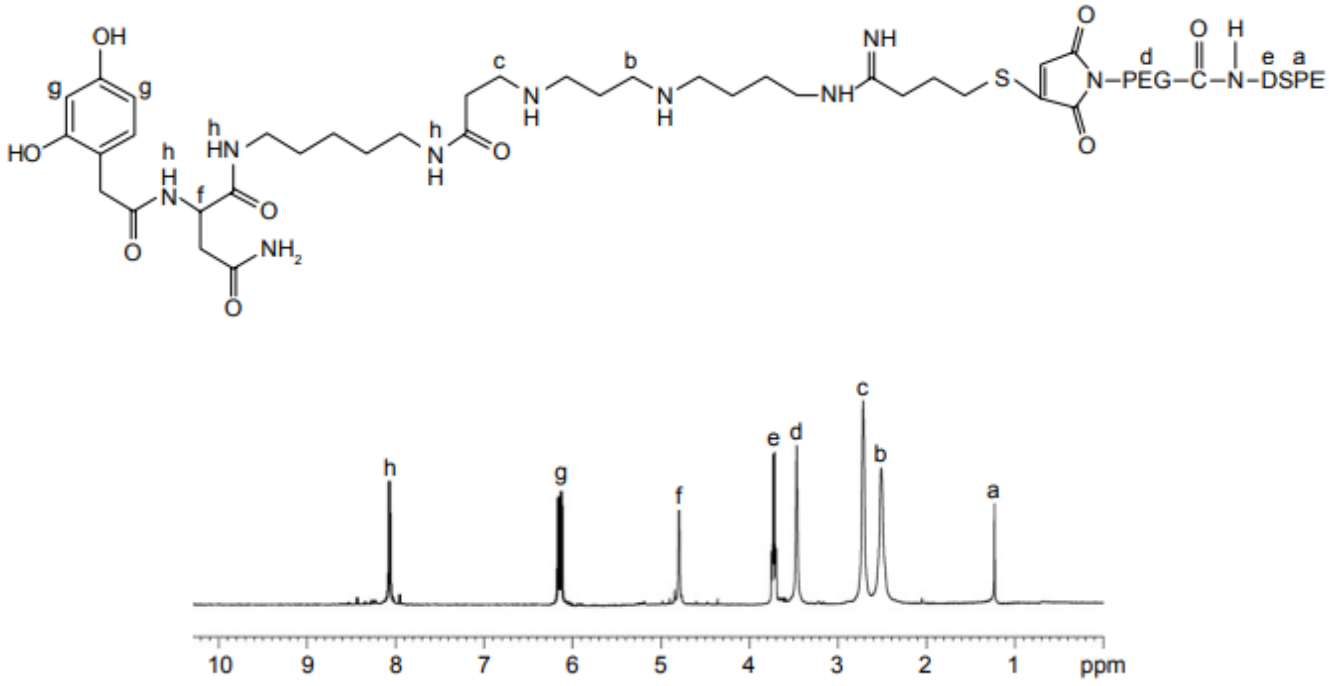

Figure 2: The chemical structure of Lf-PEG-DSPE and 1 H-NMR spectroscopy.

Preparation and characterization of NLCs

T/V-NLCs that had not been charged with any ligands were much smaller, measuring in at 96 nm, than their ligand-treated counterparts. 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

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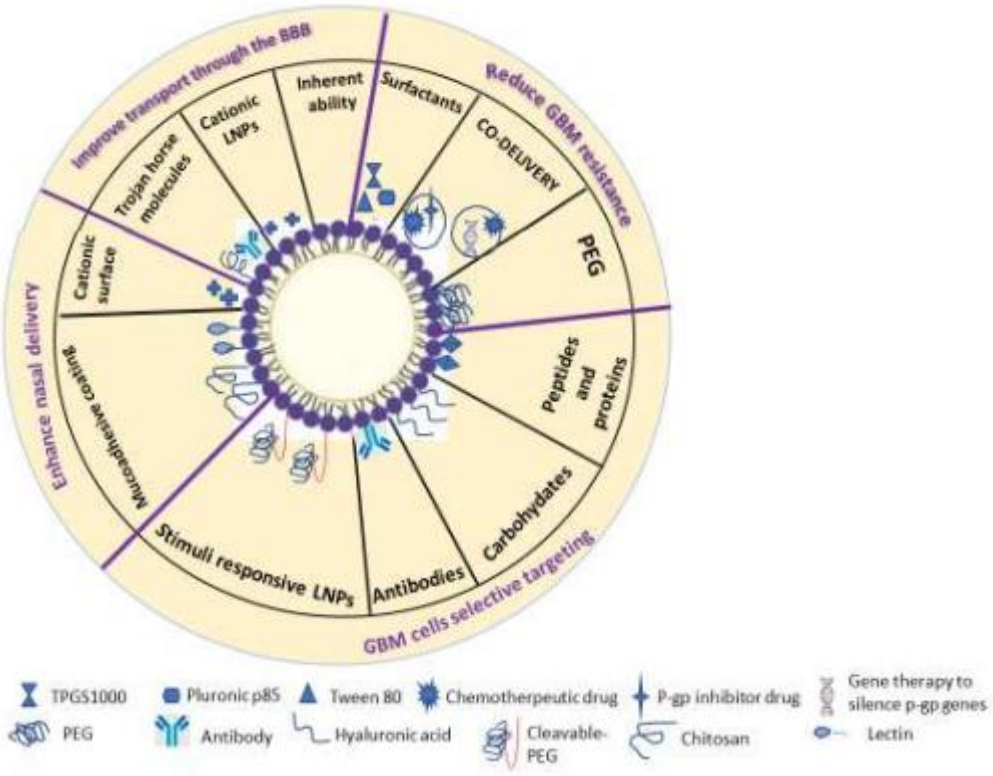

Figure 3. Modification strategies

Blood-Brain Barrier (BBB) in GBM Treatment

The blood-brain barrier (BBB) is the first major biological barrier to successful GBM therapy. It is possible that SLNs and NLCs, to a lesser degree, may cross the BBB due to their lipid makeup. This method makes use of both the AMT system route and the RMT system route. Because of its ability to bind to lipoprotein receptor-related protein 1 (LRP1) on the BBB, angiopep-2 conjugated onto the surface of nanoparticles has been proven to improve drug delivery into GBM cells. 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. Melanotransferrin is a syaloglycoprotein involved in iron absorption that is expressed in the endothelial cells of the BBB and tumor cells. Its hitherto unknown role in BBB transcytosis has been uncovered. 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) [58]. To create cationic LNPs, 3beta-[N-(N',N'-dimethylaminoethane) carbamoyl] cholesterol (DCCholestrol) was used to alter cholesterol, and then a monoclonal antibody against TfR (OX26) was attached to the resulting positively charged SLNs. OX26 conjugation and the positive surface charge of SLNs were observed to increase the bioavailability of baicalin in CSF. Malignant glioblastoma multiforme (GBM) cells may be precisely targeted using cationic SLN functionalized with anti-EGFR.

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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

Future Perspectives of SLNs and NLCs for Glioblastoma Treatment

The tumor is difficult to treat because of its diversity, resistance, aggression, and invasion, 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.

CONCLUSION

Based on our prior work, here we provide L/R-T/V-NLCs for the treatment of GBM in combination. The stability of the L/R-T/VNLCs was assured by their nanoscale size and high EE. L/R-T/V-NLCs had a number of desirable properties, features such as potent tumor suppression with low systemic toxicity, enhanced drug accumulation in tumor tissue, and increased cytotoxicity stand out. L/R-T/V-NLCs show promise as a medication delivery system for the treatment of glioblastoma.

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