A SYNOPSIS ON

DEVELOPMENT OF NANOCARRIER BASED TARGETED DRUG DELIVERY SYSTEM FOR EFFECTIVE TREATMENT OF BRAIN TUMOR

By

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Introduction

Brain tumors in the central nervous system (CNS) are the heterogenous group of primary and metastatic neoplasms characterized by poor prognosis and low patient survival rate. In another word, brain tumoris abnormal tissue mass formed due to uncontrollable multiplication of the cells in the brain and which may results into destruction of healthy cells present in the brain [1]. In United States of America (USA), the cancer related to CNS holds the second most common form of cancer in both adolescent and children; and also prime cause of tumor associated death before 40 years of age.According to GLOBOCAN 2018, nearly 296,851 new cases of brain and nervous system tumors and 241,037 deaths are diagnosed in 2018 worldwide. In India 28142 new brain tumor cases annually are reported while deaths were 24003 in 2018 [2]. The estimated incidence, mortality and 5 year prevalence of brain tumor among men in India is approximately 11,855, 9,574 and 17,251 respectively which represents 2.5%, 2.7% and 2.6% of Indian population respectively while for women it is 6976 (1.3%), 5578 (1.7%) and 10157 (1%) respectively. According to American Chemical society (ACS) facts and figure of 2020, the estimated new brain cancer cases is 23,890, however, from these, male patients are 13,590, whereas, female patients are 10,300. Additionally, the total estimated deaths due to brain cancer are 18,020, however, from these 10,190 were male patients and 7830 were female patients [3].

More than 120 types of brain tumors are identified till date and depending on the origin of tumor, most common tumors are grouped as tumors of neuroepithelial tissue, tumors of cranial and spinal nerve, tumors of meninges, heamopoitic origin neoplasm and lymphomas, tumor of sellar region, germ cell tumors and cysts [4,5].The most prevalent brain tumors are intracranial metastases from systemic cancers, meningiomas, and gliomas, specifically, glioblastoma [6,7]. Glioma is the most frequent primary brain cancer which accounts for 29% of all primary brain and CNS tumors and 80% of malignant brain tumors. These malignant gliomas are primary tumors that are derived from glial origin and account for approximately 70% of new primary brain cancer diagnosis. The classification, grading, and treatment of this diverse group of tumors have been primarily based on morphological criteria, which introduced a certain degree of interpretative subjectivity and moreover provided only suboptimal accuracy for the prediction of treatmentresponse [8]. WHO has classified glioma in three category viz. astrocytoma, oligodendrogliomas and mixed gliomas (oligoastrocyotomas). Amongst gliomas, glioblastomamultiforme (GBM) which is a grade IV astrocytoma according to the World Health Organization (WHO) classification, is the most common and aggressive form of glioma in nature [4,8]. The median survival for glioblastoma is 14 months [9]. The high mortality rate due to GBM can be attributed to specific properties of glioma which includes highly infiltrative nature and lack of clear margin. The existing therapy for GBM is nonspecific and almost fails to prevent reoccurrence of disease.

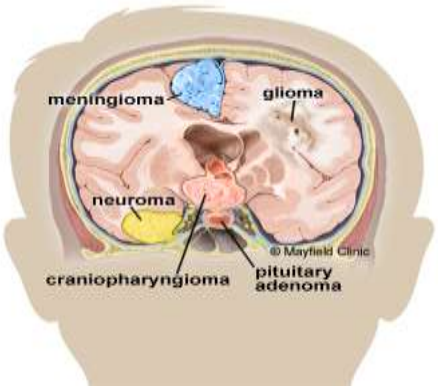

Figure 1: Brain tumor

There are currently three traditional therapies surgery, radiotherapy and chemotherapy for the treatment of brain tumor and may vary depending on the type, grade, size and location of the tumor; whether it has spread; and patient age and general health. However, these treatments have not shown up-to the mark results in the treatment of cancer. Drugs used in chemotherapy to kill tumor cells can impart toxicity to normal cells and also oxidative stress-based damage to normal cells; and also lack of tumor-specific targeting capability. In surgical operations, tumor can be surgically removed with the aid of doctors if it is identified earlier. Nevertheless, the treatment is often ineffective, costly and mostly unsuccessful, when tumor gets converted to malignant. Furthermore, the radiation utilized in the radiotherapy may lead to dermatitis, during or after treatment [10].

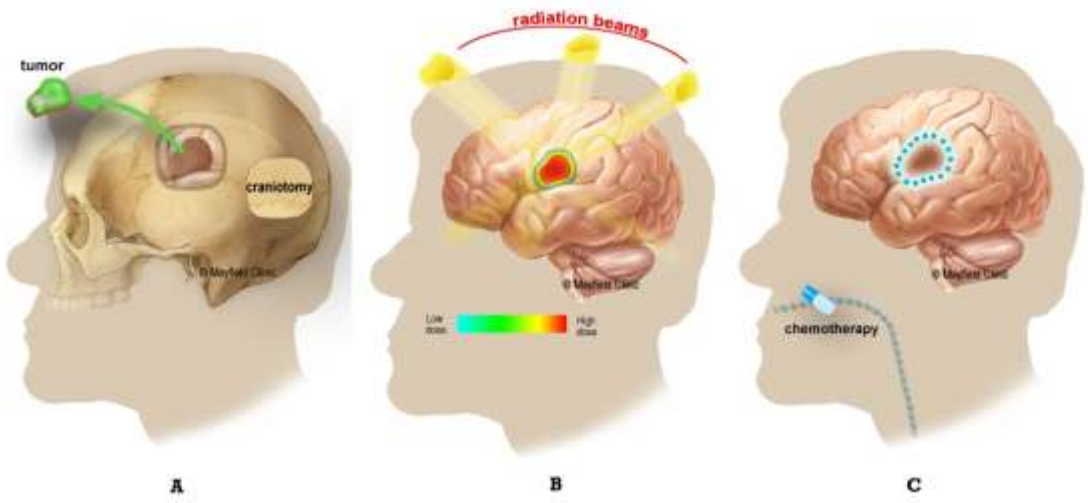

Figure 2: Treatment approaches of Brain tumor- (A) Surgery, (B) Radiation and (C) Chemotherapy

In addition, the conventional treatment has often struggled to resolve tumor microenvironment obstacles such as enhanced permeability and retention (EPR) effect, hypoxia,

acidosis and extensive angiogenesis [11]. Conventional therapy also possesses disadvantages viz. non-specific distribution of drug which may lead to toxicity to normal cells, generating resistance and also impart unwanted side effects [12]. Conventional therapeutic carrier can be removed by RES or by renal excretion easily [13]. Such disadvantages prompt the fabrication of nano-delivery system that can particularly target therapeutic moieties to tumor cells along with controlled release and targeting of therapeutic moieties to enhance intracellular localization, resulting in a reduction in non-specific toxicity of cells. Various strategies are had been recognized for therapeutic delivery to tumor sites [11]. The main priority in the production of therapies for cancer treatment is drug delivery which can target cancer which can provide therapeutic concentrations of anticancer therapeutic moieties at the action site and avoid toxicity to normal healthy cells.

Several nanoparticles with distinct biological functions have been fabricated for cancer treatment over the past three decades by modulating their physicochemical properties including composition, size, shape and surface modification. Two clinically successful targeted therapies viz. antibody-drug conjugates and nanoparticle based systems have emerged among the nanoparticles. Three antibody-drug conjugates and seven drug delivery systems based on nanoparticles targeted at a variety of human cancers have been approved at the moment. In addition, nanotechnology provides encouraging prospective for screening, formulating and administering drugs which were formerly limited due to solubility. Nonetheless, the majority of nanoparticles applied for treatment of cancer did not meet the approval of Food and Drug Administration (FDA) due to development of drug resistance, tumor relapse and failure to produce an improved anti-cancer efficacy or diagnosis and targeting. There are several nanoparticles such as protein-based nanoparticles, lipid based nanoparticles, polymeric nanoparticles and metal-based nanoparticles investigated in the treatment of cancer [14,15]. However, surface functionalization of nanoparticles with biomolecules (targeting moiety) can be a promising approach for effective targeting. Targeting moieties such as proteins, peptides, antibodies, aptamers can recognize tumor associated or tumor specific antigens in microenvironment of tumor. Ligands viz. proteins, peptides and sugars are typically more desirable than antibodies because of higher purity, higher stability, non-immunogenicity and easy to fabricate with synthetic process [16].

Recently, protein-based nanoparticles have been extensively investigated owing to their unique properties viz. ability to deliver nucleic acid, genes, peptides and proteins, both lipophilic and hydrophilic therapeutic moieties can be easily delivered, fabrication procedure is easy, during storage they show greater stability, more target specific when surface functionalized with targeting moiety and finally they are biocompatible and safe as compared to other nanoparticles [17,18]. Amongst protein-based nanoparticles, albumin was selected as carrier for exploring its utilization in the treatment of cancer. Albumin is macromolecule and mostly available in body as a plasma protein (35-50 gm/L). It is synthesized in liver with an approximately rate of 0.7 mg/h for every gm of liver (10-15 gm daily)[19,20]. It is biocompatible, biodegradable, non-

immunogenic, stable, easy to purify, water-soluble and non-toxic [21]. Again there are several types of albumin such as ovalbumin, human serum albumin and bovine serum albumin (BSA). Amongst them, BSA was considered for the research owing to their low cost, easily purified, biocompatibility, biodegradability, unusual ligand binding properties and non-toxic as compared to ovalbumin and rat albumin and BSA are widely accepted in commercial market in pharmaceutical industries [22]. Two specific methods are used in fabrication of albumin nanoparticles viz. incorporation of therapeutic or biotherapeutic moieties in albumin nanoparticles or by directly conjugating albumin/ nanoparticles to therapeutic moiety. Several biological applications of albumin conjugates are utilized as an immunohistrochemistry and immunoassay reagent, used to elucidate interactions between hormone receptors and used in the treatment of several diseases such as cancer, etc [18].

Furthermore, with the aim of specific targeting to brain tumor cells, there are several polymers, peptides, proteins are utilized as targeting moiety which are conjugated on the surface of nanoparticles. Amongst them, hyaluronic acid (HA), chondroitin sulphate (CS) and lactoferrin (Lf) are some of the targeting moieties considered in the study. HA is natural, anionic, non-sulfated glycosaminoglycan that consists of β-1,4 linked D-glucuronyl-β-(1,3) (Gln)-N-acetyl-D-glucosamine and are widely distributed throughout epithelial, neural and connective tissues [23]. HA is the largest polysaccharide in the body, with an average molecular weight of 1-8 MDa [24,25]. Human skin also contains large amount of HA i.e. 400-500 µg HA/g [26]. In other organs, the content of HA can vary from approximately from 1 to 100 µg HA/g [27]. HA plays significant role in various biological processes, cancer metastasis, cell migration, cell differentiation and wound healing [28]. Additionally, CD44, a glycoprotein, is HA receptor and are overexpressed in large number of mammalian cells and its interaction with HA is crucial for the growth and metastasis of cancer cells [29]. A lot of attention has been attracted by the researchers towards investigation of HA as a targeting moiety in cancer therapy and cancer imaging.

Similarly, CS, type of glycosaminoglycans, consists of disaccharide units of b-1,3-linked N-acetyl galactosamine and b-1,4-linked d-glucuronic acid with certain sulphated positions [30]. CS is widely distributed in mammals' skin, cartilage, bones and blood vessels [31-33]. Being similar to HA, CS has also been reported to have the ability to recognize and interact with HA-mediated CD44 receptors [34].

Secondly, Lf is an 80 kDa cationic protein belonging to transferrin family which shows 60-80% of sequence similarity with transferrin [35,36].In another way, Lf is mammalian, cationic iron-binding glycoprotein which consist of polypeptide chains of about 690 amino acids folded into two globular lobes, each of which consist of one iron-binding site [37]. The various studies demonstrated that therapeutic moiety loaded nanoparticles when conjugated with Lf were efficiently crosses BBB as compared to transferrin as targeting moiety as well as non-conjugated nanoparticles. Additionally, Lf receptors not only present on BBB but also present or overexpressed in glioblastoma cells [38,39]. The finding revealed that Lf can be a good

candidate for crossing BBB and ultimately targeting brain tumor. These fascinating results encouraged our research to fabricate HA, CS and Lf conjugated nanoparticles for enhancing brain targeting efficiency in the treatment of brain tumor.

Temozolomide (TMZ) is the first line drug used for the treatment of brain tumors. It has showed significant activity against tumor with recurrent and refractory melanoma and high grade glioma in phase I and phase II of clinical trials. Both preclinical and phase I studie suggested that after post oral administration, TMZ is completely absorbed through GIT, however, it exhibited time-dependent anti-tumor activity. TMZ with a dose of 250 mg/m2/day is administered for 5 days. On frequent dosing schedules, prominent clinical activity was observed, especially when peroral TMZ is administered once a day for 5 days for four weeks. It is basically an alkylating agent of imidazotetrazine series and a prodrug which firstly get converted into highly unstable compound 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC) at physiological pH and then MTIC is further converted into 5-aminoimidazole-4-carboxamide (AIC) and methyldiazonium ion. Formation of these methyldiazonium ions cause breakage of double strand of DNA and lead to cell cycle arrest and cell death by methylation of DNA **[40]**. Although TMZ has ability to cross blood brain barrier (BBB), it needs high systemic dose to reach therapeutic concentration in brain because of its short half-life. The previous studies proposed that continuous TMZ administration results into reduction in Nadir's platelet count. It is hypothesized, therefore, that with the help of nanoparticles, TMZ can sustain its release which will control its pharmacokinetic parameters and hence avoid repeated administration. Due to this, various systemic side effects like oral ulceration, bone marrow suppression, fatigue, vomiting, nausea and headache are associated with TMZ therapy **[41, 42].** To overcome these side effects and to improve the therapeutic activity of TMZ via targeting TMZ in brain, novel drug delivery system is required.

Furthermore, initially, Lenalidomide (LND) was proposed for the treatment of multiple myeloma, for which thalidomide is an approved medicine and LND shares structural similarities with thalidomide. It is blockbuster drug and is an immunomodulatory agent with anti-tumor and antiangiogenic properties. LND is off-white to pale-yellow powder commercialized under the trade name Revlimid. However, Revelimid hemihydrate (commercial form) due to its inadequate solubility in water has poor oral bioavailability i.e. 33%. Additionally, it has very short half-life i.e. 3 h. Several clinical investigation provide an insight for the utilization of LND in brain-tumor treatment, however clinical trial in phase II is still going on [43-45]. Thus LND with other drug makes it a possible candidate for brain tumor therapy. LND possess a limitation that it is not capable of crossing blood brain barrier (BBB) and enter cerebrospinal fluid (CSF) at concentration which are therapeutically significant. LND additionally goes with atypical biodistribution and extremely short residence time in body. LND therefore need to be administered in such a manner as to improve its brain absorption and the residence at the tumor site.

Aims and Objectives

The aim of the present research work was to develop targeted drug delivery system based on albumin nanoparticles for therapy of brain tumor. For therapy of brain tumor two model drugs were selected that were temozolomide and lenalidomide. The research work focused on development and optimization of albumin nanoparticles loaded with drug (temozolomide and lenalidomide). The optimization of nanoparticles was done on the basis of experimental design. Further for making albumin nanoparticles target specific surface modification was done with suitable targeting ligands for brain tumor targeting.

Thus the present research work was proposed to be carried out in following steps.

Step 1: Selection of suitable method for preparation of nanoparticles (albumin) based on the properties of drug.

Step 2: Optimization of prepared nanoparticles based on experimental design.

Step 3: Surface modification of nanoparticles with suitable targeting ligands and their optimization.

Step 4: Characterization and evaluation of developed nanoparticles.

Step 5: In vivo studies

Step 6: Stability studies

Summary

The present research was carried out for two different drugs (temozolomide (TMZ) and lenalidomide (LND)). Firstly temozolomide loaded nanoparticles (TNPs) were developed and optimized for brain tumor targeting. TNPs were prepared by modified desolvation method as the drug is slightly water soluble. As temozolomide shows pH dependent stability and highly susceptible to alkaline medium the method was developed such a way that drug remain stable during the formulation. Then TNPs were preliminary optimized by OVAT design to identify the critical parameters and their working range for further optimization. During preliminary optimization various parameters like drug to polymer ratio, polymer concentration, aqueous to organic ratio, crosslinker (gluteraldehyde) concentration, rate of addition of organic phase, effect of stirring speed, effect of pH, etc were screened. The results indicated that drug to polymer ratio, aqueous to organic ratio and crosslinker concentration had significant effect on quality attributes (particle size, PDI, zeta potential and entrapment efficiency) of nanoparticles. So these parameters were further optimized using Box-Behnken response surface methodology and results indicated that all three parameters had significant effect on particle size and entrapment efficiency of TNPs while non significant effect on PDI and zeta potential. In case of particle size, GA concentration showed most significant effect as compare to drug: polymer and aqueous: organic ratio on particle size. In case of entrapment efficiency, drug: polymer ratio and interaction of drug: polymer ratio and aqueous: organic ratio was having most significant effect as compare to GA concentration and aqueous: organic ratio.

After optimization of TNPs, surface modification was done with hyaluronic acid (HA) and chondroitin sulphate (CS) using carbodiimide chemistry to achieve CD44 mediated targeted delivery of TNPs (HA-TNPs and CS-TNPs). Surface modification with HA and CS was optimized on the basis of particle size, PDI, zeta potential and conjugation efficiency. Different parameters like molecular weight of HA, ligand to NPs ratio and stirring time was selected as parameter of optimization. Conjugation of HA and CS with TNPs was also confirmed by FTIR studies. For HA-TNPs development, low molecular weight HA, HA:NPs (1:1) and 30 mins of stirring was found to be optimum while in case of CS-TNPs, CS:NPs (1:1) and 12h stirring was found to be optimum to get the highest conjugation efficiency of 78.34% and 74.34% respectively.

In the next step, surface modified HA-TNPs and CS-TNPs along with TNPs were characterized and evaluated on the basis of various in vitro studies. The dynamic light scattering results indicated that hydrodynamic diameter of TNPs was within the range of 150-160 nm while in case of HA-TNPs and CS-TNPs it was increased and was in range of 200-350 nm. The increase in the hydrodynamic diameter of surface modified nanoparticles may be attributed to targeting moiety. The particle size distribution pattern indicated uniformity of nanoparticles dispersion which made them suitable for intravenous administration. Zeta potential results indicated the stability of the developed nanoparticles.

DSC study indicated that in both the cases (TNPs and surface modified TNPs) drug was molecularly dispersed in the carrier and totally encapsulated within the nanoparticles as the exothermic peak of drug was not present in the final formulations.

FTIR data also confirmed the presence of drug in the nanoparticles as all the characteristic peaks of drug was present in the developed nanoparticles with lesser intensity. FTIR data also confirmed the successful conjugation of HA and CS with TNPs as the new amide bond peak was observed in both HA-TNPs and CS-TNPs.

XRD spectrum revealed conversion of crystalline nature of TMZ in amorphous nature after encapsulation in nanoparticles as the highly intense characteristic peaks of drug was converted in the lesser intensity peaks.

TEM image of HA-TNPs and CS-TNPs showed roughly spherical shape, exhibiting a dark core surrounded by a lighter gray rim likely corresponding to the HA and CS conjugation respectively. Size of HA-TNPs and CS-TNPs obtain by TEM was lesser than obtained by zetasizer (DLS measurement). This may be due to presence of water surrounding HA-TNPs during DLS in comparison with size measured in dried state by TEM.

In vitro release data revealed biphasic release of TMZ from TNPs, HA-TNPs and CS-TNPs. All the developed nanoparticles showed initial burst release than slower and sustained release. The surface modified HA-TNPs and CS-TNPs showed more sustained release pattern of drug as compare to TNPs this may be due to slower diffusion of drug from additional HA and CS layer over the TNPs.

Results of cell viability assay of different developed nanoparticles showed higher suppression of cells as compare to pure TMZ. Surface modified TNPs (HA-TNPs and CS-TNPs both) showed higher cell suppression than TNPs. This may be due to higher uptake of the nanoparticles because of presence of targeting moiety over the surface which enhanced the cellular uptake of nanoparticles by binding with the CD44 receptor present in the surface of tumor cells.

In vitro BBB passage study also confirmed the higher permeation of nanoparticles through BBB than pure drug. This may be due to smaller size of nanoparticles than the pure TMZ. As compared to TNPs, HA-TNPs and CS-TNPs showed higher permeation this was due to the fact that CS and HA also plays role in crossing BBB. The sequence of BBB passage was as follows: HA-TNPs > CS-TNPs > TNPs > TMZ

Effect of developed nanoparticles on cell cycle arrest was also carried out and results demonstrated that as compare to pure TMZ, CS-TNPs showed higher G2/M phase cell arrest. That suggested higher inhibition of growth of cancerous cells. In case of HA-TNPs no significant changes as compare to non treated cells were observed.

Surface modification plays important role in cellular uptake of nanoparticles. HA and CS both are CD44 receptor targeting moieties. CD44 receptors are over expressed in brain tumors. To achieve the brain tumor specific targeting these CD44 receptors were targeted by HA and CS. The cellular uptake results indicated that as compared to pure drug and TNPs, cellular uptake of surface modified nanoparticles were higher in both HA-TNPs and CS-TNPs. All the developed nanoparticles were taken up by caveolae mediated pathway. CD44 receptors were also involved in the uptake mechanism and this was confirmed by the CD44 receptor blocking assay.

ROS generation in U87MG cells was estimated and the results demonstrated concentration dependent ROS generation. As the concentration of TNPs, HA-TNPs and CS-TNPs increased, an increase in ROS generation was also observed. This may be correlated with the fact that increase in concentration of TNPs, HA-TNPs and CS-TNPs led to increased concentration of released TMZ leading to increase in ROS generation. HA-TNPs and CS-TNPs showed higher ROS generation as compared to pure TMZ and TNPs. This may be due to higher uptake of HA-TNPs and CS-TNPs via CD44 receptor which led to increased TMZ concentration in the cells that ultimately caused increased ROS generation.

In vivo pharmacokinetic and biodistribution studies revealed improvement in pharmacokinetic profile and therapeutic concentration of TMZ in brain after encapsulating it in nanoparticles.

The quantitative biodistribution study of TMZ, TNPs, HA-TNPs and CS-TNPs was performed to assess the passage of TMZ through the BBB and its distribution in various vital organs viz. brain, liver, heart, lungs, kidney and spleen. The distribution of TMZ in brain tissues was approximately six folds, eight folds and nine folds higher with TNPs, HA-TNPs and CS-TNPs respectively as compared to free TMZ. This may be due to smaller size of nanoparticles as compare to pure drug and enhanced BBB permeability. As mentioned earlier HA and CS also plays role to enhance BBB permeation, presence of HA and CS over the nanoparticles facilitate the BBB crossing which led to enhance concentration of drug in the brain. The obtained results showed correlation with in vitro BBB permeation study.

Further, the results revealed significant reduction in the distribution of drug to highly perfused organs when given as nanoparticles. This may be attributed to surface modification of nanoparticles that led to prevented opsonization. After 24 h, distribution of TMZ released from HA-TNPs and CS-TNPs was more or less similar in spleen and heart whereas in liver and lungs, distribution was significantly decreased as compared to pure TMZ. Distribution of CS-TNPs was high in kidney as compared to free TMZ may be due to presence of hydrophilic polymer over the surface of TNPs

Furthermore the biodistribution and toxicity data suggested safety of developed nanoparticles as accumulation of TMZ in other vital organs decreased and no significant changes were seen in biochemical parameters of rats treated with formulations.

The interaction of synthesized nanoparticles was also assessed with respect to blood and culture media. No significant interactions were observed.

The results of stability studies indicated no significant change in particle size, % assay and zeta potential of prepared nanoparticles at both refrigerated condition and room temperature for three months which indicated their stability.

Lenalidomide loaded albumin nanoparticles (LNPs) were also prepared by desolvation method and optimized by OVAT design. Then according to optimized formula scaleup batch was prepared and subjected to lyophilization.

After optimization of LNPs, surface modification was done using lactoferrin (Lf) to achieve lactoferrin receptor targeting. As LNPs has negative surface charge and Lf has positive surface charge at neutral to slight alkaline medium, Lf is physically coated over the LNPs. For the optimization of Lf coating two different parameters like Lf concentration and time of stirring was considered and optimization was done with placebo nanoparticles on the basis of particle size and zeta potential measurement. The results indicated that 1% w/w Lf and 1hr stirring was sufficient to coated LNPs for achieving desired particle size and zeta potential. Confirmation of the coating was done on the basis of zeta potential. Initially zeta potential of LNPs was negative (-16.4 mV) which was shifted to positive side (0.9 mV) after Lf coating. Percentage coating and presence of Lf was also estimated by Bradford protein estimation assay which indicated approximate 30% coating of Lf with the LNPs. To increase the percentage of Lf coating higher concentration of Lf also tried but particle size was significantly enhanced so for preparing final optimized batch of Lf coated LNPs (LF-LNPs) 1% w/w Lf and 1hr stirring was used. After that scale up batch was prepared and lyophilized.

Surface modified Lf-LNPs along with LNPs were further characterized and evaluated on the basis of various in vitro studies. The dynamic light scattering results indicated that hydrodynamic diameter of LNPs was within the range of 120-130 nm while in case of Lf-LNPs it was increased and was in range of 145-150 nm. The increase in the hydrodynamic diameter of surface modified nanoparticles may be attributed to Lf coating. The particle size distribution pattern indicated uniformity of nanoparticles dispersion which made them suitable for intravenous administration. Zeta potential results indicated the successful coating and stability of the developed nanoparticles.

Entrapment efficiency of LND in LNPs was measured by free drug estimation and entrapment was found to be approximately 90-92% while drug loading was found to be 12%.

FTIR data also confirmed the presence of drug in the nanoparticles as all the characteristic peaks of LND was present in the developed nanoparticles with lesser intensity. FTIR data also confirmed the successful coating of Lf over LNPs.

XRD spectrum revealed conversion of crystalline nature of LND in slightly amorphous nature after encapsulation in nanoparticles as the highly intense characteristic peaks of drug was converted in the lesser intensity peaks and also confirmed presence of LND in the developed formulation.

TEM image of Lf-LNPs showed spherical shape, exhibiting a dark core surrounded by a lighter gray rim likely corresponding to the Lf coating. Size of Lf-LNPs obtain by TEM was lesser than obtained by zetasizer (DLS measurement). This may be due to presence of water surrounding Lf-LNPs during DLS in comparison with size measured in dried state by TEM as mentioned earlier.

In vitro release data revealed biphasic release of LND from LNPs. All the developed nanoparticles showed initial burst release than slower and sustained release.

Results of cell viability assay of developed nanoparticles showed higher suppression of cells as compare to pure LND. Surface modified Lf-LNPs showed higher cell suppression than LNPs. This may be due to higher uptake of the nanoparticles because of presence of targeting moiety over the surface which enhanced the cellular uptake of nanoparticles. Other cell line studies are ongoing.

After getting satisfactory results from in-vitro characterization and evaluation, formulation was subjected to animal studies. In vivo pharmacokinetic and biodistribution study was carried out in male Wistar rats. Estimation of drug in plasma and tissue homogenates is ongoing. The study related to in vivo tumor regression is also ongoing.

The results of stability studies indicated no significant change in particle size, % assay and zeta potential of prepared nanoparticles at both refrigerated condition and room temperature for three months which indicated their stability.

Ongoing work

Cell line studies

Analysis of plasma and tissue homogenates using LC-MS/MS

Tumor regression study

Conclusion

In the present work, hyaluronic acid conjugated, chondroitin sulphate conjugated and lactoferrin coated albumin nanoparticles were developed and optimized for CD44 receptor mediated and lactoferrin receptor targeted delivery of temozolomide and lenalidomide respectively. Various physicochemical properties of developed nanoparticles were investigated. All obtained results demonstrated good quality attributes in terms of particles size, PDI, zeta potential and morphology of nanoparticles. As temozolomide is highly susceptible to alkaline environment, we were able to develop such a carrier which prevent the degradation of temozolomide and enhance its stability within the carrier which can be correlated by the entrapment efficiency. In vitro release profile demonstrated the sustained release behavior of drug. In vitro cell line studies demonstrated that developed nanoparticles were able to cross blood brain barrier and reached to target tumor site by caveolae mediated endocytosis pathway. Hyaluronic acid and chondroitin sulphate enhanced the cellular uptake of nanoparticles by CD44 receptor mediated delivery which was investigated by CD44 receptor blocking assay and enhanced the therapeutic activity and performance of temozolomide towards cancerous cells as compared to pure drug. The results obtained by in vivo studies were also correlated with in vitro studies. Pharmacokinetic studies proved the enhanced concentration of temozolomide in brain when delivered by nanoparticles and toxicity data indicated safety of developed nanoparticles as no significant changes were seen in biochemical parameters of rat. In conclusion, the results of this study suggested that hyaluronic acid conjugated and chondroitin sulphate conjugated albumin nanoparticles loaded with temozolomide (HA-TNPs and CS-TNPs) has potential to substitute pure temozolomide to target brain tumor and reduce toxicity towards normal cells and may act as promising delivery platform. In case of lenalidomide, results of all in vitro studies satisfied the performance of drug within the carrier but this will further confirmed by the results of cell line and in vivo studies.

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