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Research paper

Positive-charged solid lipid nanoparticles as paclitaxel drug delivery system in glioblastoma treatment

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ABSTRACT

Paclitaxel loaded solid lipid nanoparticles (SLN) of behenic acid were prepared with the coacervation technique. Generally, spherical shaped SLN with mean diameters in the range 300–600 nm were obtained. The introduction of charged molecules, such as stearylamine and glycol chitosan into the formulation allowed to obtain positive SLN with Zeta potential in the 8–20 mV range and encapsulation efficiency in the 25–90% range.

Blood–brain barrier (BBB) permeability, tested *in vitro* through hCMEC/D3 cells monolayer, showed a significantly increase in the permeation of Coumarin-6, used as model drug, when vehicled in SLN. Positive-charged SLN do not seem to enhance permeation although stearylamine-positive SLN resulted the best permeable formulation after 24 h.

Cytotoxicity studies on NO3 glioblastoma cell line demonstrated the maintenance of cytotoxic activity of all paclitaxel-loaded SLN that was always unmodified or greater compared with free drug. No difference in cytotoxicity was noted between neutral and charged SLN.

Co-culture experiments with hCMEC/D3 and different glioblastoma cells evidenced that, when delivered in SLN, paclitaxel increased its cytotoxicity towards glioblastoma cells.

1. Introduction

Glioblastoma multiforme (GBM) is the most common form of primary brain tumor in the central nervous system (CNS); its aggressive nature and evasiveness to treatments make it one of the most lethal cancers [1]. Current treatments for GBM provide a tumor surgical resection followed by pharmacotherapy and radiotherapy. Pharmacotherapy, directed by residual tumor cells elimination, ranges from common chemotherapeutic agents such as temozolomide to more recent anti-angiogenic agents and immunotherapeutic treatments [2]. However, anti-cancer therapeutic agents have not significantly increased the median survival of GBM patients over the past 10 years. The 5-year survival rate of GBM patients after treatment that includes surgical resection, radiation and chemotherapy, is 9.8%.

The failure of chemotherapy is due to the inability of intravenously administered anticancer agents to reach the brain

Abbreviations: PTX, paclitaxel; SLN, solid lipid nanoparticles; BA, behenic acid; ST, stearylamine; GCS, glycol chitosan; BBB, blood–brain barrier; GBM, glioblastoma multiforme; CNS, central nervous system; NP, nanoparticles; CBSA, cationic bovine serum albumin; NHEJ, non-homologous end joining; PK, protein kinase; CS, chitosan; Cou-6, coumarin 6; Na-BA, sodium behenate; CHOL, cholesterol; FD, freeze-dried; DMEM, Dulbecco's modified Eagle's medium; NS, neurosphere; AC, adherent cell; ATM, ataxia telangiectasia mutated; ChK2, checkpoint kinase; p-53BP1, 53 binding protein 1; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; IF, immunofluorescence; HR, homologous recombination; TEM, transmission electron microscopy; EE, entrapment efficiency; Pgp, P-glycoprotein; MRP1, multidrug resistance related protein 1.

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parenchyma. An endothelial cell monolayer associated with pericytes and astrocytes, known as the blood–brain barrier (BBB), separates blood from the cerebral parenchyma and prevents the penetration of drugs into the CNS.

BBB is a functional unit composed by the peculiar endothelium of brain microvessels, the capillary basal lamina and the surrounding astrocytes, neurons, microglial cells and pericytes, which contribute to the maintenance of the barrier properties [3], Fig. A.1]. The presence of tight junctions and adherent junctions between adjacent endothelial cells [4], the lack of fenestrations and pinocytotic vesicles [5], the abundance of efflux transporters belonging to the ATP binding cassette family on the endothelium luminal side [6] account for the low delivery of drugs, such as antineoplastic agents [7], from the bloodstream to the brain parenchyma.

Various invasive strategies have been developed to improve the penetration of drugs into the brain [8]. Traditional approach to overcome brain drug delivery obstacles includes direct intracerebral drug injection [9], which is associated with a high risk for the patient.

Less invasive strategies have also been investigated. One approach consists in generating a transient disruption of BBB in conjunction with the systemic administration of anticancer agents. The intracarotid administration of a hyperosmotic solution such as mannitol led to a rapid diffusion of fluid across the cerebral endothelium, moving out of the endothelial cells into the vascular lumen and inducing the opening of the tight junctions for a few hours [10].

Another approach concerns the modification of drugs in order to make them more lipophilic, improving their penetration into the brain by passive diffusion. Lipophilic analogs and prodrugs were thus developed [11].

A more recent strategy to deliver drugs to the brain is the use of colloidal polymers to form nanometer sized carriers [12]. The basic reason of common acceptance of these vehicles is due to their drug release controlled profile of as well as to their selected targeting mechanism. Targeting action may be due to the steric hindrance created by nano-vectors: after parenteral administration, due to steric phenomenon they conceal themselves from opsonization event induced by tissue macrophages. By this way they achieve targeting ability to the brain and partially avoid other reticuloendothelial system organs like liver, spleen, etc. [13].

From the last few decades, nanoparticles (NP) have attracted considerable interest in targeting drug molecules to the brain [14]. The correct mechanism of barrier opening by NP is not exactly known; the delivered NP enter into the brain by crossing the BBB by various endocytotic mechanisms, as polymeric albumin or poly(butyl cyanoacrylate) NP are reported to enter into the brain by their small size mediated endocytosis [15,16]. An increased drug retention in brain blood capillaries combined with an adsorption to capillary can increase drug transport due to an enhanced concentration gradient; an increase of BBB fluidization, an opening of tight junctions between endothelia, and an inhibition of the P-glycoprotein efflux system are other possible mechanisms that can increase brain drug concentration [17].

Although NP may be designed to entrap high molecular weight or hydrophilic therapeutics, BBB retardation of drug NP entrapped is based on NP characteristics and not on the therapeutic agent [18].

Indeed, poly(butyl cyanoacrylate) NP overcoated with 1% polysorbate 80 have been experimentally successful as brain drug delivery for doxorubicin [19] and dalargin [20], poly(lactic-co-glycolic acid) and cetyl alcohol/polysorbate NP for paclitaxel brain delivery [21,22].

In addition to BBB functional characteristics limiting permeation, brain microvasculature endothelia also present a luminal electrostatic barrier at physiologic pH. The negative electrostatic charge is created by surface expression and adhesion of the glycocalyx residues: proteoglycans, sulfated mucopolysaccharides, and sulfated and sialic acid-containing glycoproteins and glycolipids [23]. Cationic molecules have been shown to occupy anionic areas at the BBB endothelium [24] and increase BBB permeability via a presumed tight junction disruption [25].

Recent *in vitro* reports have demonstrated that positive-charged NP have an increased brain distribution compared to anionic and neutral NP [26]. However, there is little data regarding brain permeability of positive-charged NP. Lu et al. [27] developed and evaluated cationic bovine serum albumin (CBSA) conjugated with poly(ethyleneglycol)–poly(lactide) NP (CBSA–NP). To evaluate the effects of brain delivery, BSA conjugated with pegylated NP (BSA–NP) was used as the control group and Coumarin-6 was incorporated into the NP as the fluorescent probe. The qualitative and quantitative results of CBSA–NP uptake experiment compared with those of BSA–NP showed that rat brain capillary endothelial cells took in much more CBSA–NP than BSA–NP at 37 °C, at different concentrations and time incubations. After a dose of 60 mg/kg CBSA–NP or BSA–NP injection in mice caudal vein, fluorescent microscopy of brain coronal sections showed a higher accumulation of CBSA–NP in the lateral ventricle, third ventricle and periventricular region than that of BSA–NP. In an experimental work Lockman et al. [15] evaluated the effect of neutral, anionic and cationic charged NP on BBB integrity and NP brain permeability. Neutral NP and low concentrations of anionic NP had no effect on BBB integrity, whereas, high concentrations of anionic NP and cationic NP disrupted the BBB. The brain uptake rates of anionic NP at lower concentrations were higher than of neutral or cationic formulations at the same concentrations.

In the literature, many authors studied solid lipid nanoparticles (SLN) as drug delivery systems to deliver drugs to the CNS [28,29]. SLN are disperse systems having size ranging from 1 to 1000 nm which represent an alternative to polymeric particulate carriers and are composed of physiological or biocompatible lipids or lipid molecules with a history of safe use in therapy and are generally suitable for intravenous administration.

As few data are present in the literature about positive-charged SLN for brain delivery, the purpose of this work will be to prepare, characterize and evaluate *in vitro* the potential of positive-charged SLN to vehicle paclitaxel (PTX) to the brain for GBM treatment.

PTX, a diterpene isolated from *Taxus brevifolia*, is one of the most active chemotherapeutic agents against a wide panel of solid tumors including urothelial, breast, lung, and ovarian cancers. It has been demonstrated that PTX is effective against glioblastoma cells *in vitro* [30,31], however its clinical use is limited due to its poor BBB penetration capability and drug-resistance [32,33].

Due to its low water solubility, PTX is formulated in a mixture of Cremophor® EL and dehydrated ethanol (50:50 v/v) a combination known as Taxol®. However, Taxol® has some severe side effects related to Cremophor® EL and ethanol [34]. Therefore, there is an urgent need for the development of alternative PTX formulations.

Recently, a new solvent-free technique, defined as ‘‘coacervation’’, was developed to prepare fatty acids-based SLN [35]. Briefly, a fatty acid alkaline salt micellar solution in the presence of an appropriate polymeric stabilizer was prepared; when the pH is lowered by acidification, the fatty acid precipitates as nanoparticles owing to proton exchange between the acid solution and the sodium salt.

SLN were prepared using two different positive-charged substances: ST or GCS. ST possesses a hydrocarbonic chain that can probably be incorporated within the lipid matrix, while its positive charge is exposed to the external surface. GCS, a chitosan derivative conjugated with ethylene glycol branches, is a water soluble at a neutral/acidic pH values polymer whose pendant glycol