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Transferrin-conjugated nanoparticles of Poly(lactide)-D-α-Tocopheryl polyethylene glycol succinate diblock copolymer for targeted drug delivery across the blood–brain barrier

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ABSTRACT

We developed in this research a nanoparticle system for targeted drug delivery across the blood–brain barrier (BBB), which consists of the transferrin (Tf) conjugated nanoparticles of poly(lactide)-D-α-Tocopheryl polyethylene glycol succinate (PLA-TPGS) diblock copolymer. The NPs were prepared by the nanoprecipitation method and characterized for their various physicochemical and pharmaceutical properties. Cellular uptake and cytotoxicity of the Tf-conjugated PLA-TPGS NPs formulation of coumarin 6 as a model imaging agent or Docetaxel as a model drug were investigated in close comparison with those for the PLGA NPs formulation, the bare PLA-TPGS NPs formulation as well as with the clinical Taxotere®. The Tf-conjugated PLA-TPGS NPs formulation demonstrated great advantages over the other two NPs formulations and the original imaging/therapeutic agents. IC50 data showed that the Tf-conjugated PLA-TPGS NPs formulation of Docetaxel could be 23.4%, 16.9% and 229% more efficient than the PLGA NPs, the PLA-TPGS NPs formulations and Taxotere® after 24 h treatment, respectively. Moreover, our preliminary *ex vivo* biodistribution investigation demonstrated that although not as satisfactory, the Tf-conjugated PLA-TPGS NPs formulation could be able to deliver imaging/therapeutic agents across the BBB.

1. Introduction

Diseases related to the brain such as malignant tumors, cerebral malaria, multiple sclerosis, HIV-dementia, stroke, Alzheimer's and Parkinson's diseases have long been a challenging area for both of the clinical practitioners and the medical researchers. In oncology, there has been little breakthrough since the past few decades in terms of increasing the average life span of patients suffering from brain cancers. Astrocytomas, oligoastrocytomas and oligodendrogliomas are the three most common types of brain tumors diagnosed in adults [1,2]. According to National Cancer Data Base (NCDB), prognosis has been poor with overall 5-year survival rate of only 30% for patients with astrocytomas [3]. The main obstacle in brain cancer treatment is the presence of blood–brain barrier (BBB), which selectively regulates and limits the amount and types of chemotherapeutic agents permeating into the brain parenchyma [4]. In fact, human brain contains more than 100 billion of capillaries which comprise four cell types, namely endothelial cells, pericytes, astrocytes and vascular nerve ending cells. It is the lining of the brain capillary endothelial cells (BCEC), which forms the tight junctions and express efflux transport properties of the BBB that restrict and control the exchange of nutrients or bioactive substances between the peripheral bloodstream and the central nervous system (CNS) [5–7]. This barrier acts as a unit to protect the brain exogenous materials which could potentially damage the brain tissues. Nearly all the drugs, particularly neuropharmaceuticals, cannot penetrate the BBB. Only drugs which are highly lipophilic or small enough in size can usually enter the brain [8–10].

To solve the problem of drug delivery across the BBB, the conventional invasive strategies such as interventricular or intracerebral (transcranial) delivery and temporary impairment of the BBB are no longer feasible in consideration of the cost-benefit ratio for patients. They are often associated with high risk of infection, limited delivery area, drug diffusive resistance from interstitial fluid, high pre- and post-surgical cost or even serious clinical side effects in the case of high-dose therapy [11–13].

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Therefore, non-invasive approaches are imminent to be discovered and developed in view of the advantages of manipulating endogenous transport systems to overcome the BBB without physically interfering with the brain tissues which otherwise may cause medical complications.

Almost in all circumstances, essential nutrients such as glucose, amino acids and vitamins for proper brain functioning are required and the general route of absorbing these nutrients is through the BBB [14,15]. As a result, specific receptors (biomarkers) on the BBB responsible for transporting the nutrients are crucial in governing the nutrient entry to and from the brain tissues. Of various categories in endogenous transport systems, receptor-mediated transport (RMT) is an attractive strategy due to a few reasons such as circumvention of the multi-drug resistant (MDR) P-glycoprotein (P-gp) efflux transporter in the BBB and cancer cells, high specificity in ligand-receptor binding, low immunological response, unrestricted to cargo size and the ability to transport macromolecules such as proteins and peptides across BBB [6,16,17]. Some RMT receptors found in the BBB include insulin, leptin, insulin-like growth factor, transferrin, and low-density lipoprotein (LDL) [18–21]. It is these endogenous transport systems that serve as the platform for developing new drug formulations that can reach to the brain by exploiting these highly selective molecular carriers. Among the receptors found to date, transferrin receptors are known to be expressed in the luminal membrane of capillary endothelium of the BBB [6,22,23]. This receptor is also over-expressed in many types of malignant tumors as well as in human tissues of liver, intestinal epithelium and erythroblasts, but the level of expression is nearly undetectable in other normal, non-dividing healthy tissues [22,24–28]. Therefore, transferrin has been widely studied and shown to be a promising molecular probe for targeted drug delivery to the brain.

Produced mainly by the liver, transferrin (Tf) is a single polypeptide glycoprotein of approximately 80 kDa consisting of about 679 amino acids [29]. It plays key roles in multiple biological functions of human body, which include co-factor in DNA replication, antimicrobial effects against pathogenic bacteria, prevention of tissue damage by toxic effects of free iron and iron transport for cell growth or differentiation through binding of one Fe^{3+} to each N-lobe and C-lobe of transferrin [27,29,30]. The iron-loaded Tf then interacts with its corresponding receptors in the BBB to induce the blood-to-brain receptor-mediated pathway to deliver iron to the central nerve system (CNS) [17,23,31,32]. Although there was controversy over the exact mechanism of the BBB entry of iron-bound Tf, some studies have suggested that most of the Tf were able to be transcytosed by endosome across the BBB without undergoing intraendothelial degradation [20,33,34]. It might be this reason that allows iron-bound Tf to be subsequently endocytosed by TfR − expressing brain malignant tumors, for instance, to meet the rapid cell proliferative activity. As a result, various drug carriers employing Tf as a trojan to gain selective access to the CNS have been reported. Some examples of drugs delivered by this pathway include paclitaxel and daunorubicin as cancer therapeutic agents, quinine dihydrochloride and artemisinin as anti-malarial medications [16,35–37]. Hence, development of nanoparticulate carrier of biodegradable polymers in combination with transferrin as a brain-targeting ligand for treatment of the brain diseases is the backbone of this study.

There are a few basic important criteria that need to be carefully considered in designing such drug delivery devices across the BBB. Firstly, these nanocarriers loaded with therapeutic drugs often have to be small in size. The favorable range is about 100–200 nm in diameter in order to enhance permeability and retention (EPR) of the nanocarriers due to the leaky vasculature of tumor microenvironment and to avoid human complement system, macrophage uptake, splenic as well as liver filtration [38–40]. Secondly, the surface of the nanocarriers has to be sufficiently hydrophilic in order to minimize the adsorption of opsonins such as immunoglobulin, complement product C3b and fibronectin [41,42]. Attachment of the opsonin triggers opsonization process of macrophages and subsequently removal of nanocarriers from the body. A common technique to avoid opsonins is to incorporate poly (ethylene glycol) (PEG)-containing compounds into the nanoparticulate system to create surface water-bound layer having low affinity to opsonins, thus enhancing circulation time of the devices in blood before they reach the targeted sites [43]. Together with these two criteria, targeting ligands could be attached/conjugated to decorate the nanocarriers to achieve optimal targeting efficiency. Some examples of BBB targeting have been reported, which include surface modification by coating with non-ionic, PEG-containing surfactants such as polysorbate 80 and poloxamers or with BBB targeting ligands such as apolipoproteins, peptides and growth factors on the nanoparticles of biodegradable polymers such as poly(lactide-co-glycolide) (PLGA) and poly(butyl cyanoacrylate) (PBCA) [2,12,44–46]. Although more precise engineering of targeted nanodevices can be achieved through chemical conjugation of endogenous ligands to the surface of polymeric nanoparticles, physical adsorption of such ligands still represents a much simpler and milder approach which may otherwise reduces the bioactivity of the ligand to be bound to its corresponding receptor [11,47].

As previously demonstrated, the poly(lactide)-D-alpha-tocopheryl polyethylene glycol 1000 succinate (PLA-TPGS) diblock copolymer has been shown as a potential candidate for nanoparticle formulation of anticancer drugs such as Paclitaxel and Docetaxel [48,49]. Its amphiphilic structure can achieve high drug loading as well as better stabilize the nano-suspension generated during the fabrication process than many other homopolymers and hydrophobic copolymers. The exposure of TPGS on the nanoparticles surface also help enhance the cellular uptake of the nanoparticles as shown in the *in vitro* experiments and increase absorption and circulation time in animal models. The objective of this research is to develop a drug delivery system across the BBB, which is the PLA-TPGS nanoparticle formulation with transferrin surface modification to target specifically to the brain for brain cancer treatment. Firstly, the PLA-TPGS nanoparticles formulation of Docetaxel as a model anticancer drug with Tf surface modification is fabricated. Secondly, the characteristics of the active targeted nanoparticles (ATN) are compared with non-targeting nanoparticles (NTN) and the widely studied PLGA nanoparticles. Thirdly, *in vitro* cellular study is carried out using C6 glioma cell line or the BCEC as an *in vitro* model of the BBB to determine the targeting ability of transferrin-modified PLA-TPGS nanoparticles. Finally, *ex vivo* biodistribution, especially that in the brain and blood, of the transferrin-modified PLA-TPGS nanoparticles versus the non-targeting nanoparticles are demonstrated.

Table 1
Characterization of non-targeted and targeted nanoparticles loaded with Docetaxel.

Formulation	Size[a] (nm)	Polydispersity[a]	Zeta potential[a,d] (mV)	EE[b,c] (%)
PLGA	161.5 ± 1.5	0.108 ± 0.027	−25.9 ± 6.2	31.14 ± 1.56
PLA-TPGS	121.6 ± 1.4	0.137 ± 0.010	−36.5 ± 0.5	79.77 ± 2.59
PLA-TPGS/Tf	137.6 ± 5.1	0.147 ± 0.026	−31.1 ± 9.8	73.48 ± 2.28

[a] $n = 6$.
[b] $n = 3$.
[c] EE = (amount of drug loaded in nanoparticles/total amount of drug added during fabrication) × 100%
[d] Measurement done in deionized water at pH = 7.0.