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An injectable acellular matrix scaffold with absorbable permeable nanoparticles improves the therapeutic effects of docetaxel on glioblastoma

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ABSTRACT

Intratumoral drug delivery (IT) is an inherently appealing approach for concentrating toxic chemotherapies at the site of action. However, for most chemotherapies, poor tumor penetration and short retention at the administration site limit their anti-tumor effects. In this work, we describe permeable nanoparticles (NPs) prepared with a novel amphiphilic polymer, RRR-α-tocopheryl succinate-grafted-ε-polylysine conjugate (VES-g-ε-PLL). The nanoparticles (NPs) of VES-g-ε-PLL exhibited an ultra-small hydrodynamic diameter (20.8 nm) and positive zeta potential (20.6 mV), which facilitate strong glioma spheroid penetration ability in vitro. Additionally, the hydrophobic model drug docetaxel (DTX) could be effectively encapsulated in the nanoparticles with 3.99% drug loading and 73.37% encapsulation efficiency. To prolong the retention time of DTX-loaded nanoparticles (DTX-NPs) in the tumor, intact decellularized brain extracellular matrix (dBECM) derived from healthy rats was used as a drug depot to adsorb the ultra-small DTX-NPs. The intact DTX-NPs-adsorbing dBECM scaffold was further homogenized into an injectable DTX-NPs-dBECM suspension for intratumoral administration. The DTX-NPs-dBECM suspension exhibited slower DTX release than naked DTX-NPs without compromising the tumor penetration ability of DTX-NPs. An antitumor study showed that the DTX-NPs-dBECM suspension exhibited more powerful in vitro inhibition of tumor spheroid growth than free DTX solution or DTX-NPs. Due to strong tumor penetration ability and prolonged retention, DTX-NPs-dBECM led to complete suppression of glioma growth in vivo at 28 days after treatment. The therapeutic mechanism was due to enhanced proliferation inhibition and apoptosis of tumor cells and angiogenesis inhibition of glioma after treatment with DTX-NPs-dBECM. Finally, the safety of DTX-NPs-dBECM at the therapeutic dose was demonstrated via pathological HE assay from heart, liver, spleen, lung and kidney tissues. In conclusion, permeable nanoparticle-absorbing dBECM is a potential carrier for intratumoral delivery of common chemotherapeutics.

1. Introduction

Glioblastoma multiforme (GBM) is the most prevalent and lethal form of malignant brain tumor and is considered to be one of the deadliest human cancers [1]. As a first treatment, most cancer patients receive surgery to remove as much of the tumor as possible. The poor prognosis associated with GBM is mostly due to poorly defined GBM tumor borders, incomplete resection of the tumor and tumor recurrence after surgical resection [2]. After surgery, systemic or local chemotherapy or radiotherapy is performed to

Abbreviations: GBM, glioblastoma; DTX, Docetaxel; BBB, blood-brain barrier; dBECM, Decellularized brain extracellular matrix; PLL, Polylysine; IT, Intratumoral drug delivery; IFP, Interstitial fluid pressure; VES, Vitamin E succinate; MRI, Magnetic Resonance Imaging.

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eliminate residual cancer and prevent the recurrence of carcinogenesis.

Docetaxel (DTX) is a potent anticancer drug that appears to be 2- to 4-fold more potent than paclitaxel [3]. However, similar to many chemotherapeutic agents, DTX has a fairly low solubility in water and a poor ability to penetrate the blood brain barrier (BBB), which results in its rapid clearance from the blood and low drug accumulation inside the GBM following either systemic therapeutic efficacy [4]. Moreover, even if transported across BBB and into tumor tissue, it is difficult to for a low dose of the drug to penetrate through the deeper tumor tissue and into the core of the tumor because of elevated interstitial fluid pressure (IFP) and the abnormal extracellular matrix (ECM) of solid tumors. In addition, the short retention time of DTX in the tumor is also an important factor that results in its low therapeutic effect against GBM because it is easily cleared from the tumor. Therefore, new therapeutic strategies are required to ensure efficient tumor penetration of this drug and prolong its retention time inside tumor tissues [5].

Intratumoral drug delivery (IT) is an inherently appealing approach for concentrating toxic chemotherapies at the site of action. This mode of administration is currently used in a number of clinical treatments such as neoadjuvant, adjuvant, and even standalone therapies when radiation and surgery are not possible [6]. Ideally, IT administration could result in even drug distribution throughout the tumor, and the drug could be retained for sufficient time to act primarily at the tumor site. Unfortunately, drug distribution in the tumor is often non-uniform, and the drug quickly cleared, primarily due to poor drug penetration through the stiff tumor extracellular matrix and poor drug retention in the presence of outward fluid pressure gradients [7,8]. Drug retention within the tumor can be enhanced by encapsulating the drugs in nanoparticles or hydrogels [6]. Unfortunately, loading drugs into macromolecules only exacerbates their poor distribution inside the bulk tumor because these larger constructs tend to result in highly limited tumor penetration abilities even when enhanced with pressure-driven flow [9,10]. Thus, a delivery system is required that can enhance both retention and distribution of drugs following IT administration.

Recently, selected nanocarriers (such as liposomes or polymer micelles) were modified with functional materials to give them optimal physicochemical characteristics such as suitable particle size, zeta potential and surface hydrophobicity, etc., for improved tumor penetration. For example, it was reported that nanosystems with dimensions ranging from 10 to 100 nm and with positive surface charges should be able to access and disseminate within the tumor after parenteral administration [11]. The cationic particles, which are self-assembled from a triblock copolymer of poly (D,L-lactide-*co*-glycolide)-*block*-branched polyethyleneimine-*block*-poly(D,L-lactide-*co*-glycollide), penetrated into the multicellular spheroids against a hydraulic pressure gradient via active transport mechanisms [12]. Similarly, several studies have suggested the great potential of cationic nanoparticles for intratumoral penetration [13,14]. These cationic nanoparticles were prepared using a synthetic copolymer with high molecular weight, which usually resulted in a larger particle size of greater than 50 nm. A number of studies demonstrated that nanosystems with diameters less than 50 nm exhibited enhanced solid tumor penetration ability via passive diffusion compared with those with diameters larger than 100 nm [15,16]. However, it was also observed that particles less than 25 nm were cleared more rapidly from tumor tissues than 60 nm particles, which led to a short retention time inside the entire tumor [17]. Therefore, it is necessary to develop an effective drug delivery system that is capable of prolonging the retention of the ultra-small cationic nanoparticles inside the tumor and does not compromise their tumor penetration ability.

The extracellular matrix (ECM) is produced by extracting the cells and soluble matrix components from the extracellular matrix, and thus, it exhibits almost all of the properties of normal tissue and maintains a low potential for inflammatory attack on the graft because most antigenic proteins are extracted from the tissue. Because of its porous network scaffold, ECM has been commonly used as a novel biomaterial to control the release of macromolecular therapeutics [18,19]. Moreover, previous studies showed distinct differences between the compositions of brain ECM and other tissue ECM, such as spinal cord or urinary bladder matrix. The brain ECM-retained scaffold-related composites include collagen type IV, laminin, and fibronectin, and glycosaminoglycans but contain relatively small amounts of nutritional components [20]. Because of its inherent three-dimensional ultra-structure, dBECM scaffolds that mimic the native extracellular matrix can sequester certain growth factors for sustained release of these therapeutics [21]. Although ECM has been described as a platform for the sustained release of macromolecular therapeutics in the literature, few publications have reported the application of ECM as a carrier of small molecular chemotherapeutics. The incompatibility between these hydrophobic chemotherapeutics and hydrophilic ECM was a major reason for the limited application of ECM in the field of small molecular therapeutics delivery.

Recently, our group synthesized a novel ε-polylysine-based graft polymer, RRR-α-tocopheryl succinate-grafted-ε-polylysine conjugate (VES-g-ε-PLL). Based on its smaller molecular weight, the amphiphilic VES-g-ε-PLL conjugate could self-assemble into cationic nanoparticles (NPs) with a size of less than 30 nm and might exhibit stronger tumor penetration ability than larger nanoparticles. Additionally, because of the superior cell penetration and adhesive ability of the ε-PLL backbone, it was expected that the cationic VES-g-ε-PLL particles could exhibit enhanced penetration ability toward glioma in situ [22]. These permeable VES-g-ε-PLL nanoparticles were intended for use as carriers of hydrophobic chemotherapeutics to improve their solubility in water. To prolong the retention of these ultra-small nanoparticles inside the tumor post-intratumor injection, these cationic NPs were further absorbed into dBECM scaffolds through electrostatic interaction to prepare an injectable NPs-dBECM complex. Compared with other scaffolds, such a strategy allows the nanocarrier system to mimic cell behaviors such as cell adhesion and cell migration in the extracellular matrix, which could benefit the retention and penetration of nanocarrier systems in tumor tissues. It was hypothesized that the permeable NPs might carry the encapsulated drug and penetrate into the depth of the tumor.

For proof of concept, in this study, the model drug DTX was first encapsulated into cationic nanoparticles of VES-g-ε-PLL to prepare DTX-loaded nanoparticles (DTX-NPs). Second, the DTX-NPs were adsorbed into the intact dBECM scaffold, and the intact DTX-NPs-dBECM scaffold was further homogenized into an injectable DTX-NPs-dBECM suspension (Fig. 1). The in vitro tumor spheroid penetration ability and sustained release profile of DTX-NPs-dBECM were carefully investigated. The in vivo antitumor effect and tumor penetration of this injectable DTX-NPs-dBECM complex were also thoroughly studied. Finally, the molecular mechanism of the therapeutic effect of this DTX-NPs-dBECM complex was also explored.

2. Materials and methods

2.1. Materials

DTX was purchased from Knowshine (Shanghai, China). *N*-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxy-succinimide (NHS), poly(lysine) and RRR-α-tocopherol