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Development of a hypoxia-triggered and hypoxic radiosensitized liposome as a doxorubicin carrier to promote synergetic chemo-/radio-therapy for glioma

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ABSTRACT

The treatment of malignant primary brain tumors is challenging. Concomitant radiochemotherapy has become the standard clinical treatment for malignant glioma, but there are two critical challenges to overcome in order to increase efficacy. First, glioma is known to have increased resistant to radiation due to its intra-tumoral hypoxia. In addition, the blood-brain barrier (BBB) restricts the distribution of the chemotherapeutic agent to the brain. Therefore, we developed a hypoxic radiosensitizer-prodrug liposome (MLP), in order to deliver DOX to the tumor and to overcome the above challenges, achieving a synergistic chemo-/radiotherapy treatment of malignant glioma. In this study, hypoxic radiosensitizer nitroimidazoles were conjugated with lipid molecules with a hydrolysable ester bond to form MDH. MDH was mixed together with DSPE-PEG2000 and cholesterol to make MLP liposomes, which were found to have strong radiosensitivity and to promote cargo release under hypoxic conditions, due to the properties of nitroimidazoles under hypoxic conditions. MLP/DOX was found to have distinct advantages, including precise and stealthy pharmacokinetics and efficient passive uptake by the tumor. Furthermore, the combination of MLP/DOX and radiotherapy (RT) significantly inhibited glioma growth as assessed by *in vivo* bioluminescence imaging. These findings suggest that MLP is a promising candidate as a DOX delivery system to enhance the antitumor treatment effects on glioma, owing to synergistic chemo-/radiotherapy.

1. Introduction

Malignant gliomas are the most common of all malignant primary brain tumors [1,2]. Despite aggressive therapy with surgery, chemotherapy, and radiation, the median survival time is only approximately 12 months [3]. While concomitant radiochemotherapy has become the standard treatment [4,5], there are two aspects of malignant gliomas that complicate therapeutic efficacy. Firstly, glioma tumors are understood to be more resistant to radiation, because of their intra-tumoral hypoxia. Secondly, the BBB restricts the dissemination of chemotherapy from the blood to the brain [6–8]. Taken together, it is clear that the development of new approaches is necessary, in order to improve radiochemotherapy for glioma treatment.

RT and chemotherapy are two important therapeutic modalities for all cancers. The combination of the two has been shown to achieve optimum and synergistic treatment against glioma [9,10], however the oxygen deficiency, or hypoxia, present in the core part of the glioma is one of the major causes for RT failure in these cases [11]. Accumulating evidences show that the hypoxia makes glioma

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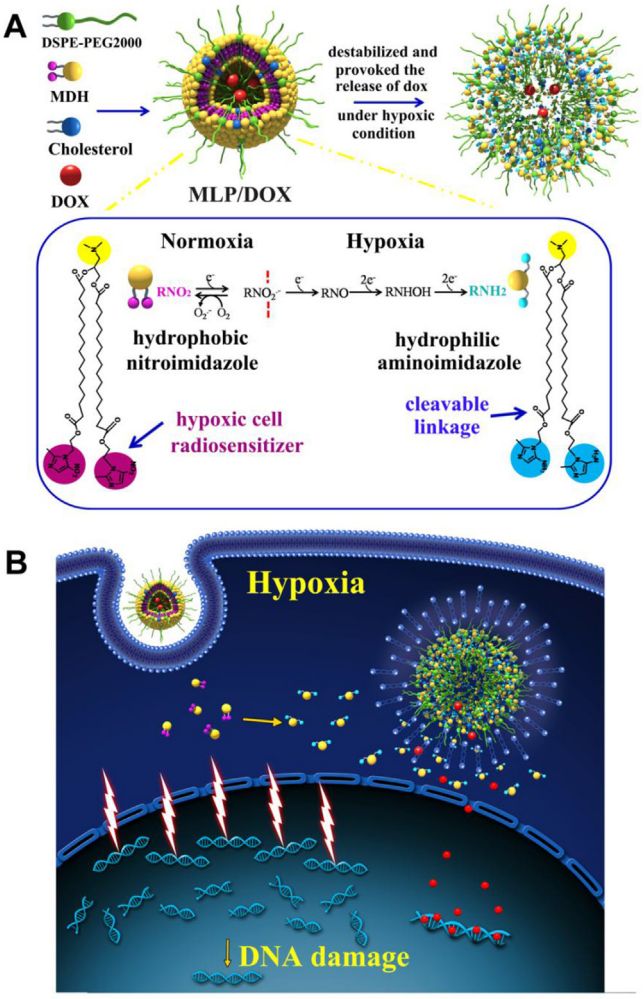
tumoral cells radioresistant and that the extent of hypoxia in malignant gliomas before RT is closely correlated with the patient's overall survival time [12,13]. Therefore, the development of efficient and safe hypoxic radiosensitizers has become an urgent priority of cancer therapeutics. Nitroimidazoles have been most widely utilized in the development of hypoxia-responsive drug carriers, because the hydrophobic nitroimidazoles convert to hydrophilic aminoimidazoles via a series of selective bioreductions when presented with hypoxic conditions [14,15]. Meanwhile, nitroimidazoles have been proposed as hypoxic cell radiosensitizers, since they have been found to mimic the effects of oxygen on the radiochemical process [16,17]. 2-Methyl-5-nitroimidazole-1-ethanol (MI, Metronidazole) is a specific hypoxic cell radiosensitizer and is used in controlled trials to evaluate the possible enhancement of radiation effects in patients with malignant glioma [18–21]. However, high-dose MIs and the subsequent neurological toxic effects have limited their clinical application [22,23]. Therefore, the major challenge in using nitroimidazoles to render hypoxic cells radiosensitive is the development of a method for evaluating the drug concentration in the tumor and minimizing toxic side effects to normal tissues.

Doxorubicin (DOX) is a very widely used anticancer drug and is crucial for the treatment of various types of cancers through induction of DNA damage and inhibition of the progression of the enzyme topoisomerase II [24–26]. DOX has very little effect when used in glioma therapy, however, since it cannot cross the BBB, and thus cannot achieve sufficient concentrations in glioma cells [27–29]. The vascular endothelial cells and associated pericytes are often abnormal in tumors, and in brain tumors, some of the BBB architecture is destroyed [30,31], allowing nanoparticles to cross the BBB. To take advantage of this phenomenon, researchers have studied a variety of nanoparticles, such as polymers, micelles, and liposomes, in order to facilitate DOX delivery [32–38]. Liposomes have been intensively explored and have been successful in drug delivery applications. Liposomal DOX has been shown to be safe and has moderate activity that might lead to long-term stabilization in recurrent high-grade glioma patients, since the liposome increases the efficiency of DOX delivery across the BBB [39,40]. Unfortunately, chemotherapy alone does not appear to work as well for patients with gliomas. Recently, combination of different treatment methods has been considered as a promising strategy to enhance the therapeutic efficiency and versatile anticancer drug co-delivery systems have received tremendous attention [41]. Therefore, to develop a nanoplatform to simultaneously deliver radiosensitizers and chemotherapy drugs to tumor can improve chemoradiotherapy synergistic therapy.

To overcome these limitations, we introduced RT sensitive liposomes that are responsive to hypoxia as a novel DOX delivery system, in order to synergistically inhibit the growth of brain glioma tumors (Scheme 1). In this study, an MDH lipid molecule was synthesized, consisting of MIs as the hydrophobic tails and tertiary amines as the hydrophilic head group. We designed the MDH lipid molecules for the following purposes: 1) The MDH combined with 1, 2-distearoyl-*sn*-glycero-3-phosphoethanol amine-*N*-methoxy-poly (ethylene glycol 2000) (DSPE-PEG2000) and cholesterol can prepare the liposome (MLP) to load DOX (MLP/DOX) for the inhibition of glioma growth. 2) Tertiary amines are highly pH dependent, so when used as the MDH hydrophilic head at a low pH, with high cationic charge, they can enhance tumor cellular uptake of MLP. However, at pH 7.4, they can reduce the positive charge density on the surface of the MLP to increase the blood circulation half-life. 3) MI is poorly soluble in water, but its reductive derivative, aminoimidazole, is highly water-soluble. Based on this unique feature of the nitroimidazole group, we hypothesize that the lipid-bilayer would destabilize, provoking the release of DOX under hypoxic conditions. 4) MI has a strong effect on sensitization to RT [42,43]. These features make MLP an excellent carrier for the specific delivery of DOX in concurrent chemotherapy and RT therapy. In this study, the combination of chemotherapy and RT for glioma treatment was also investigated, both *in vitro* and *in vivo*.



Scheme 1. Schematic of the hypoxia-triggered, RT sensitive liposome (MLP) drug-delivery system. A) Formation and mechanism of MLP/DOX. B) Schematic illustrating MLP/DOX applications for the hypoxic cell radiosensitizer and its hypoxia-induced release of DOX into the cell nuclei to achieve synergistic chemo-/radiotherapy glioma treatment.

After tail vein injection, MLP/DOX was able to penetrate the BBB and enter into the glioma tumor due to the enhanced permeability and retention (EPR) effect. It was then internalized into the cells by endocytosis. Hypoxia can induce hydrophobic nitroimidazoles to convert to hydrophilic aminoimidazoles through the transfer of six electrons [44]. Due to their electron affinity, MIs increased the radiosensitivity of radio-resistant hypoxic cells, enhancing the DNA damage induced by ionizing radiation. Meanwhile, MLP/DOX conjugated with MI-grafted MDH lipid molecules was expected to destabilize and provoke the rapid release of DOX when present in a hypoxic environment. The released DOX would theoretically accumulate in the nuclei to kill the tumor cells, and when combined with MLP's radiosensitization effects, to enhance the synergetic chemo-/Radio-therapy against glioma (Scheme 1B).