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Low molecular weight heparin mediating targeting of lymph node metastasis based on nanoliposome and enzyme–substrate interaction

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ABSTRACT

The aim of our study is to develop a new function of low molecular weight heparin (LMWHEP) for targeting tumor metastatic lymph node based on LMWHEP-modified nanoliposome and LMWHEP–heparanase (HPA) interaction (LMWHEP–HPA). At First, LMWHEP-modified nanoliposomes (LMWHEP-LPs) were prepared by the electrostatic attraction and the physiochemical properties were evaluated. Then the effects of LMWHEP–HPA on the stability and drug release were investigated. In addition, the cellular uptake of LMWHEP-LPs was studied by using Hela, MCF-7, L929 and RAW264.7 cells. Finally, the targeting ability as well as the tissue distribution was examined in the mice model bearing Hela tumor lymph node metastasis. LMWHEP-LPs prepared had suitable physicochemical properties. The effect results of LMWHEP–HPA showed that LMWHEP coated on the surface of nanoliposome could be degraded by HPA. Compared with the unmodified-nanoliposome, the LMWHEP modification could improve the cellular uptake and increase the targeting ability to the metastatic lymph nodes according to LMWHEP–HPA. This study demonstrates LMWHEP is a highly promising polymer material for the targeting of tumor lymph node metastasis.

1. Introduction

The lymphatic system plays an active role in the cancer metastasis (Dadras et al., 2005; Ji, 2006; Koyama et al., 2008). Many malignant tumors metastasize first to the lymph nodes via the lymphatic (Ogawa et al., 2014; Yoshihara et al., 2013). Therefore, the lymph node metastasis has been a key factor of the cancer staging and prognosis (Rinderknecht & Detmar, 2008; Stacker, Baldwin, & Achen, 2002). In recent years, extensive investigations have been carried out in the area on the prediction, diagnosis and treatment of tumor lymph node metastasis (Terwisscha van Scheltinga et al., 2014). In the aspect of treatment, the knowledge of the growth factors which stimulate tumor lymphangiogenesis, such as VEGF-C/VEGF-D/VEGFR-3, provides new targets for potential molecular therapy to prevent or decrease lymph node metastasis (Inagaki et al., 2014). However, in clinical practice, chemotherapy still is the preferred treatment method for the lymph node metastasis. Conventional systemic chemotherapy cannot effectively be delivered to the lymphatic system without dose-limiting toxicities. While the drug delivery systems which specifically and selectively target drug to metastatic lymph nodes or tumor cells are warranted to address the current gaps. As the drug delivery systems of chemotherapy drugs, nanoliposomes offer advantages in improving the drug concentration in lymph nodes, extending the duration time of action and decreasing drug amounts into blood circulation (Cai, Yang, Bagby, & Forrest, 2011). But nanoliposome has no selectivity between normal lymph node and metastatic lymph node. This problem can be resolved by the surface modification of nanoliposomes which has been proven as an effective strategy for potential targeting nanoliposome to metastatic lymph node (Yan et al., 2012).

Heparanase (HPA) is an endoglycosidase which cleaves heparan sulfate proteoglycan (HSPG) at specific sites to release heparan sulfate (HS) fragments as well as proteins that are trapped within the extracellular matrix (ECM), and hence HPA participates in the degradation and remodeling of the ECM (Nakajima, Irimura, Di Ferrante, & Nicolson, 1984; Reiland et al., 2004). The activity

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of HPA closely correlates with tumor invasion and metastasis (Parish, Freeman, & Hulett, 2001), and the enzyme is preferentially expressed in metastatic tumor cells. Several studies have shown that highly metastatic tumor cells produce high amounts of HPA compared to their poorly metastatic or non-metastatic cells (Jin, Nakajima, & Nicolson, 1990; Nakajima, Irimura, & Nicolson, 1986, 1988). The study also shows that the expression of HPA significantly increased in the metastatic popliteal lymph node but not in the primary tumor by observing changes of MR contrast enhancement (Dafni et al., 2005). Thus HPA activity is directly bound up with metastatic tumor cells and lymph node metastasis.

Low molecular weight heparin (LMWHEP), a negative-charged, biodegradable and water-soluble natural polysaccharide belonging to glycosaminoglycans, is the first choice of effective anticoagulants for thrombosis treatment (Ihaddadene, Le Gal, Delluc, & Carrier, 2014), which have been commercially available like bemiparin (Ciccone et al., 2014) and enoxaparin (Ingle & Agarwal, 2014). The potential anticancer effects of anticoagulants were first reported in 1954. Both unfractionated heparin (UFH) and LMWHEP, especially LMWHEP, show the biological functions of antitumor and anti-metastasis. And the apparent benefit of LMWHEP with a better prognosis is demonstrated in the studies in patients with metastatic (Smorenburg & Van Noorden, 2001) or locally advanced cancer (Klerk et al., 2005). One of the mechanisms for LMWHEP reducing the tumor metastasis is the heparanase (HPA) inhibition (Lapierre et al., 1996; Vlodavsky et al., 1993). Glycol-splitting of LMWHEP chain generates flexible joints along the HPA, resulting in close interaction and effective inhibition of the heparanase enzyme (Vlodavsky et al., 2007). And LMWHEP can be degraded by the heparin-degrading activity of HPA, demonstrated by in vitro (Lee, Lee, Bae, & Park, 2010) and in vivo (Nilasaroya, Martens, & Whitelock, 2012) studies. But LMWHEP as a medium used for targeting the tumor metastatic lymph node via the drug delivery system modification and the substrate (LMWHEP)–enzyme (HPA) interaction has not been investigated previously.

With these considerations, low molecular weight heparin (LMWHEP) is firstly used to target the lymph node metastasis. We hypothesize that LMWHEP modified nanoliposomes as the drug delivery system could enhance targeting efficacy of lymph node metastasis based on the following strategies (Scheme 1): (1) LMWHEP as a negative-charged and water-soluble polysaccharide can coat on the surface of positively-charged nanoliposome and promote lymphatic drainage of LMWHEP-LPs from interstitial sites into the lymphatic circulation. (2) In the metastatic lymph nodes, LMWHEP can bind with HPA which is secreted by metastatic tumor cells, leading to improving and increasing metastatic lymph node retention and uptake. (3) LMWHEP degraded by HPA improves the cellular uptake as the result of enhancing targeting effect on metastatic tumor cell.

2. Material and methods

2.1. Material

LMWHEP (enoxaparin/enoxaparin sodium) was purchased from Shanghai Yiji Industrial Co., Ltd., China. Soybean phospholipids (LIPOID S100, SPC) were purchased from Germany Lipoid GmbH Co. Ltd., Germany. Cholesterol (CHOL) was purchased from Tianjin Bodi Chemical Holding Co., Ltd., China. Dimethyldioctadecylammonium Bromide (DDAB) was supplied by Tokyo Chemical Industry Co., LTD., Japan. Docetaxel (DTX), the purity of which was over 99%, was purchased from Lianyungang Gabriel Biochemical Technology Co., Ltd., China. Coumarin-6 (C6), the purity of which was over 99%, was purchased from Shanghai Fortune Bio-tech Co., Ltd, China. Taxotere® was supplied by Sanofi-Aventis Group, France. Soybean phospholipids (LIPOID S100, SPC) were purchased from Germany Lipoid GmbH Co. Ltd., Germany. 4′,6-diamidino-2-phenylindole (DAPI) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM) and RPMI-1640 were purchased from Gibco (BRL, MD, USA). All other chemicals were of reagent grade and were obtained commercially. Human cervical cancer cells (HELA), human breast cancer cells (MCF-7), mouse fibroblast cell (L929) and mouse monocyte-macrophage cells (RAW264.7) were obtained from Chinese Academy of Sciences (Shanghai, China).

2.2. Type and preparation method of LMWHEP

The enoxaparin as a low molecular weight heparin (LMWHEP) was used and studied in this research, complying with the requirements of European Pharmacopoeia (EP) 7.0 (European Pharmacopoeia, 2010). Enoxaparin was commercially available LMWHEP which was produced by alkaline depolymerisation of the benzyl ester derivative of heparin from porcine intestinal mucosa (EP7.0).

2.3. Preparation of LMWHEP-LPs

DTX-loaded nanoliposomes (DTX-LPs), C6-loaded nanoliposomes (C6-LPs), positively-charged DTX-loaded nanoliposomes (+DTX-LPs) and positively-charged C6-loaded nanoliposomes (+C6-LPs) were prepared, respectively, for different uses by thin-film hydration and sonication method. The prepared nanoliposomes and their lipid compositions were as follows: (1) DTX-LPs as the control; CHOL:DDAB:DTX =35:26:2:4 (molar ratio); (2) C6-LPs for CLSM; SPC:CHOL:C6 = 35:26:0.04 (molar ratio); (3) +C6-LPs for conjugating LMWHEP; SPC:CHOL:DDAB:C6 = 35:26:2:0.04 (molar ratio); (4) +DTX-LPs for conjugating LMWHEP; SPC:CHOL:DDAB:DTX = 35:26:2:4 (molar ratio). DDAB with bromide group was used as the cationic molecule. The experimental condition of +DTX-LPs was detailly described as follows. A mixture of SPC/cholesterol/DDAB/DTX (35/26/2/4, by mM) were dissolved in chloroform and dried into a thin film by a rotary evaporator (RE52CS, Shanghai Yarong Bio-Chem Instruments, China). The film was desiccated under vacuum overnight, and hydrated with distilled water at 50 °C water baths. The resulting solution was sonicated at 400 W for 5 min, and then filtered through the 0.8 μm filter membrane. DTX-LPs, C6-LPs, +C6-LPs were prepared by the above procedure.

LMWHEP-modified DTX-loaded nanoliposomes (LMWHEP-LPs) and LMWHEP-modified C-6-loaded nanoliposomes (LMWHEP-LPs/C6) were prepared by thermostat-titration method according to electrostatic attraction. Briefly, LMWHEP solution (distilled water, 10 mg/ml) were kept at 25 °C with 20 rpm magnetic stirring. +DTX-LPs or +C6-LPs were slowly dropped into an above LMWHEP solution (1:1, V/V) under vigorous stirring. After completion of the drop-wise addition, stirring was continued for 10 min. A gel chromatography column (1.0 cm × 10 cm) was filled with SephadexG-50 (GE Healthcare, US) which was applied to separate the liposomal systems, un-entrapped drug and free LMWHEP. Finally, the fresh nanoliposomes prepared were lyophilized using a freeze dryer (FD-1, Boyikang, China).

2.4. Characterization of LMWHEP-LPs

The morphologies of nanoliposomes were investigated using transmission electron microscopy (TEM) (JEM-1200EX, JEOL, Japan). The thermodynamic characters of following samples were analyzed, respectively, by a DSC generator (Dsc1STARe, Mettler Toledo, Switzerland): LMWHEP, DTX, lyophilized powder of DTX-LPs, lyophilized powder of LMWHEP-LPs and the physical mixture