Acta Biomaterialia 31 (2016) 186–196

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

A dual strategy to improve the penetration and treatment of breast cancer by combining shrinking nanoparticles with collagen depletion by losartan

Xingli Cun, Shaobo Ruan, Jiantao Chen, Li Zhang, Jianping Li, Qin He *, Huile Gao *

Key Laboratory of Drug Targeting and Drug Delivery Systems, West China School of Pharmacy, Sichuan University, No. 17, Block 3, Southern Renmin Road, Chengdu 610041, China

ARTICLE INFO

Article history:
Received 1 September 2015
Received in revised form 12 November 2015
Accepted 1 December 2015
Available online 7 December 2015

Keywords:
Losartan
Size-shrinkable nanoparticles
Tumor extracellular matrix
Deep tumor penetration

ABSTRACT

Although development of nanomedicines has been a promising direction in tumor treatment, the therapeutic outcome of current nanomedicines is unsatisfying, partly because of the poor retention and penetration in tumors. Recently, a kind of tumor microenvironment sensitive size shrinkable nanoparticles (DOX-AuNPs-GNPs) has been developed by our lab, which could enhance the tumor penetration and retention depending on the size shrinking. However, the further enhancement is still restricted by dense collagen network in tumors. Thus in this study, we combined DOX-AuNPs-GNPs with losartan to deplete tumor collagen (constituted up to 90% of extracellular matrix) to further improve tumor penetration. *In vitro*, DOX-AuNPs-GNPs can shrink from over 117.8 nm to less than 50.0 nm and release DOX-AuNPs under the triggering of tumor overexpressed matrix metalloproteinases-2 (MMP-2). *In vivo*, pretreatment with losartan significantly decrease the collagen level and improve the tumor penetration. In combination, losartan combined with DOX-AuNPs-GNPs showed the best drug delivery efficiency, striking penetration efficiency and best 4T1 breast tumor inhibition effect. In conclusion, this study provided a promising synergetic strategy to improve the tumor treatment efficiency of nanomedicines.

Statement of significance

We have developed a dual strategy for deep tumor penetration through combining size shrinkable DOX-AuNPs-GNPs with depleting tumor collagen by losartan. Additionally, we demonstrate therapeutic efficacy in breast tumor bearing mouse model. DOX-AuNPs-GNPs co-administration with losartan is a novel and highly attractive strategy for anti-tumor drug delivery with the potential for broad applications in clinic.

1. Introduction

In the past decades, nanomedicines, owing to their significant advances of elongated drug circulation time, minimized systemic toxicity and enhanced therapeutic effectiveness over conventional medicine, have offered new opportunities for cancer treatment [1]. More than 20 nano-scaled therapeutics have been approved by the FDA for clinical use, but the overall curative effect is still modest due to the poor tumor penetration and retention [2]. As a result, the drug concentration in tumor center was much lower than the effective dose, leading to poor tumor therapy efficiency [3].

The tumor microenvironment is structurally heterogeneous, containing irregular leaky vasculature and abnormal lymphatic vessels [4]. The abnormal structure leads to accumulation of fluid and proteins in the interstitium, resulting in the elevation of the interstitial fluid pressure (IFP) [3]. Besides, tumor extracellular matrix (ECM), which provides structural support for tissue architecture, becomes stiffer and thicker due to increased amounts of collagen synthesis and deposition [5]. These factors together induce the tumor interstitial hypertension, which can significantly hinder the penetration of nanomedicines in tumor [3]. Overcoming these physiological barriers is a major challenge for the field of nanomedicines research. Several strategies have been developed to enhance the penetration of nanomedicines through reducing the tumor interstitial hypertension [6–10], for instance, using

* Corresponding authors.
E-mail addresses: qinhe@scu.edu.cn (Q. He), gaohuile@scu.edu.cn (H. Gao).

http://dx.doi.org/10.1016/j.actbio.2015.12.002
1742-7061/

anti-VEGF antibodies or inhibitors to normalize tumor vasculature, and using TGF-β blockade to prevent the formation of abnormal lymphatics vessels [11]. Another method is using collagen production inhibitors to target the tumor ECM, such as enzyme, collagenase or losartan for reducing the tumor density and destroying the collagen lattice in tumor [12]. The majority of ECM contains high content of collagen, which is widely spread in solid tumor tissue, including breast cancer [13–15]. So effectively reducing the collagen level in tumor may increase the penetration of nanomedicines.

And it has been widely proved that losartan could reduce the collagen level in solid tumors via an angiotensin II type I receptor, which could mediate down regulation of tumor transforming growth factor-β1 (TGF-β1) [16,17]. Thus in this study, losartan was used to decrease the collagen level of tumor to improve penetration of particles.

Particle size was considered the most critical factor affecting the penetration and retention in tumor interstitium [18–20]. Generally, the tumor permeability of nanoparticles decreases when particle size increases [21]. Small particles (about 20 nm) such as gold nanoparticles (AuNPs) displayed relatively low accumulation around tumorous tissues due to the high permeation rate and the subsequent clearance into surrounding tissues. Thus, small nanoparticles penetrate deeply into the tumor tissue but can't retain long [19]. On the other hand, large nanoparticles (about 100 nm) are suitable for the enhanced permeability and retention (EPR) effect, which benefit from slower migration rate through the interstitial space, but they can't reach the deep region of tumor, because of the large size [21]. Therefore, a nanocarrier that can shrink from large size to small size in tumor may achieve both high retention and deep penetration. Consequently, DOX-AuNPs-GNPs, a kind of size shrinkable nanoparticles, has been established through fabricating small sized doxorubicin (DOX) loaded gold nanoparticles (DOX-AuNPs) onto matrix metalloproteinase-2 (MMP-2) degradable gelatin nanoparticles (GNPs) in our previous work [22]. The nanoparticles could passively accumulate in tumor and reduce their size by MMP-2 degradation to facilitate transportation in tumor.

However, the size shrinkable DOX-AuNPs-GNPs has enhanced the penetration and retention in tumor tissue, the penetration of nanoparticles into deep tumor is still hindered by physiological barriers such as collagen networks which is major constructed by Collagen I [23]. To further enhance the deep penetration in tumor tissues, we combined the size-shrinkable DOX-AuNPs-GNPs with losartan pretreatment, as illustrated in Fig. 1.

In this study, the strategy of combined losartan with size-shrinkable DOX-AuNPs-GNPs was developed and evaluated by its tumor microenvironment-responsive size shrinking ability and DOX releasing rate. The level of Collagen I was determined by Masson's trichrome staining assay and immunofluorescence staining. And tumor penetration efficiency was evaluated by *ex vivo* image, confocal microscopy imaging and transmission electron microscope (TEM). Tumor treatment was also carried out on Collagen I expressed 4T1 xenograft bearing mice model [24]. After the treatment, HE staining and TUNEL staining were used to evaluate tumor cell apoptosis.

2. Materials and methods

2.1. Materials

Doxorubicin Hydrochloride was obtained from Beijing Huafeng United Technology Co., Ltd (Beijing, China). Thiol-polyethylene glycol (PEG-SH, MW = 5000) was obtained from Laysan Bio Inc (Arab, USA). Auric chloride acid was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Losartan was obtained from Meilun Biological Technology Co., Ltd (Dalian, China). Rabbit collagen type I polyclonal antibody was purchased from Proteintech, Inc. (Chicago, USA). Rabbit CD34 polyclonal antibody was obtained from Immunoway Biotechnology Co. (Newark, USA). Alexa Fluorescence 594-conjugated donkey anti-rabbit secondary antibody and Cy3-conjugated donkey anti-rabbit secondary antibody were purchased from Jackson ImmunoResearch Laboratories, Inc (West Grove, USA). 4′-6-Diamidino-2-pheylindole (DAPI) and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Beyotime Institute Biotechnology (Haimen, China). 1-[3-(Dimethylaminopropyl)]-3-ethylcarbodiimide Hydrochloride (EDC) and N-hydroxy-succinimide (NHS) were purchased from Keddia Reagent (Chengdu, China). The 4T1 cells were obtained from Chinese Academy of Sciences Cell Bank (Shanghai,

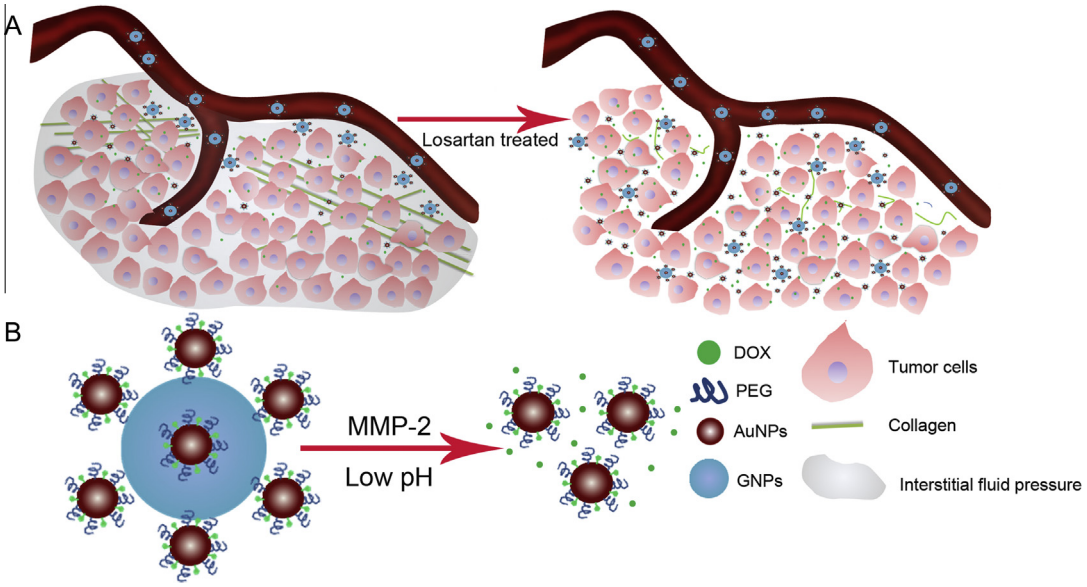

Fig. 1. Elucidation of the strategy. (A) Losartan treatment reduced the collagen level of 4T1 tumor, leading to deeper penetration of DOX-AuNPs-GNPs. (B) MMP-2 triggered DOX-AuNPs-GNPs shrunk and low pH induced DOX release in tumor.