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Original Article

Captopril improves tumor nanomedicine delivery by increasing tumor blood perfusion and enlarging endothelial gaps in tumor blood vessels

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ABSTRACT

Poor tumor perfusion and unfavorable vessel permeability compromise nanomedicine drug delivery to tumors. Captopril dilates blood vessels, reducing blood pressure clinically and bradykinin, as the downstream signaling moiety of captopril, is capable of dilating blood vessels and effectively increasing vessel permeability. The hypothesis behind this study was that captopril can dilate tumor blood vessels, improving tumor perfusion and simultaneously enlarge the endothelial gaps of tumor vessels, therefore enhancing nanomedicine drug delivery for tumor therapy. Using the U87 tumor xenograft with abundant blood vessels as the tumor model, tumor perfusion experiments were carried out using laser Doppler imaging and lectin-labeling experiments. A single treatment of captopril at a dose of 100 mg/kg significantly increased the percentage of functional vessels in tumor tissues and improved tumor blood perfusion. Scanning electron microscopy of tumor vessels also indicated that the endothelial gaps of tumor vessels were enlarged after captopril treatment. Immunofluorescence-staining of tumor slices demonstrated that captopril significantly increased bradykinin expression, possibly explaining tumor perfusion improvements and endothelial gap enlargement. Additionally, imaging *in vivo*, imaging *ex vivo* and nanoparticle distribution in tumor slices indicated that after a single treatment with captopril, the accumulation of 115-nm nanoparticles in tumors had increased 2.81-fold with a more homogeneous distribution pattern in comparison to non-captopril treated controls. Finally, pharmacodynamics experiments demonstrated that captopril combined with paclitaxel-loaded nanoparticles resulted in the greatest tumor shrinkage and the most extensive necrosis in tumor tissues among all treatment groups. Taken together, the data from the present study suggest a novel strategy for improving tumor perfusion and enlarging blood vessel permeability simultaneously in order to improve nanomedicine delivery for tumor therapy. As captopril has already been extensively used clinically, such a strategy has great therapeutic potential.

Introduction

Nanomedicine drug delivery systems have become main stream in tumor treatment [1]. To achieve a tumoricidal effect, nanomedicines have to undergo three processes to reach tumor cells: transport in the blood, transvascular transport and interstitial transport [2,3]. However, complex tumor microenvironment including poor and heterogeneous perfusion, unfavorable vessel permeability and a high density of stromal cells, as well as increased interstitial pressure always compromises the effective delivery of nanomedicines into tumor cells and, consequently, their therapeutic benefits [4,5]. This partially explains the suboptimal clinical outcomes for some food and drug administration (FDA) approved nanomedicines such as pegylated liposomal doxorubicin (Doxil/Caelyx) and nanoparticle albumin-bound paclitaxel (Abraxane) [6,7] for solid tumor treatment. So, tumor

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microenvironment modification has been widely explored as an important tool to improve nanomedicine delivery for tumor treatment [8,9].

In tumors with abundant blood vessels, agents including chloroquine, dopamine and vascular endothelial growth factor (VEGF) inhibitors et al., have been used to normalize tumor vessels by increasing the coverage rate of pericytes on endothelial cells, in order to improve tumor perfusion and so enhance drug delivery [10–12]. However, this would only benefit the delivery of free drugs *in vivo* or relatively smaller nanomedicines (20–40 nm) rather than larger ones around 100 nm [13,14]. A defined period of one or two weeks is always necessary for vascular normalization and a judicious dose of vascular normalizer was also a requirement in order to prevent the excessive pruning of tumor vessels [15]. To analyze the microenvironment of tumors with abundant vessels further, it has been found that in a typical tumor a low level of extracellular matrix and stromal cells exist and tumor cells are always in the vicinity of tumor vessels [16,17]. Therefore, nanomedicines could reach tumor cells after effective extravasation from tumor vessels. So, low perfusion and unfavorable vessel permeability pose the main obstacles to nanomedicine delivery in these tumors. It is well documented that there remains a great deal of room for tumor perfusion [18,19] and vessel permeability improvements [20–22] to enhance nanomedicine delivery. It follows that strategies aimed at improving tumor perfusion and simultaneously enhancing endothelial gaps are of great experimental and clinical interest.

Captopril is an angiotensin-converting enzyme inhibitor (ACEI), and is used widely in clinics to control hypertension [23,24]. It acts by inhibiting the activity of angiotensin-converting enzyme (ACE) and so reducing the expression of the potent pressor agent, angiotensin II, and increasing the levels of the powerful vasodilator, bradykinin (BK) [25,26], therefore dilating blood vessels and reducing blood pressure [27–29]. These established effects of captopril on blood vessels as well as its upregulation of BK led us to speculate that captopril may dilate tumor blood vessels to improve tumor perfusion, and increase the endothelial gaps in tumor capillaries due to the increased expression of BK as an inflammatory mediator at the site of tumors [29,30]. If this is so, captopril has the potential to enhance nanomedicine delivery to tumors and could represent a useful modulating agent.

This is the first time that the widely used drug captopril has been used to improve tumor perfusion and enlarge endothelial gaps simultaneously and so enhance nanomedicine delivery to tumor for therapy (Fig. 1). Glioma, which has abundant blood vessels [19], was chosen as the tumor model and nanoparticles (NPs) based on FDA-approved materials including polyethyleneglycol (PEG)-polylactic acid (PLA) were used as the nanomedicine for modeling. To determine the effects of captopril on the tumor microenvironment, including tumor perfusion and vessel permeability, experiments using laser doppler imaging (LDI), lectin-labeling, and the scanning electron microscope (SEM) were carried out. The expression of BK, established to be the downstream signaling moiety of captopril was analyzed by immunofluorescence staining. In addition, the effect of captopril treatment on delivery of NPs *in vivo* was assessed by fluorescence imaging and investigations of frozen tumor slices. Finally, the therapeutic efficacy of captopril treatment combined with paclitaxel (PTX)-loaded NPs (NPs-PTX) was evaluated with pharmacodynamic experimentation.

Materials and methods

Materials

Captopril was ordered from Dalian Meilun Biotech Co., Ltd (Dalian, China). Courmarin-6 was from Sigma (USA). DyLight® 488-labeled tomato lectin (Lycopersicon esculentum) came from Vector (USA). Sodium cholate was purchased from the Shanghai Chemical Reagent Company. Hoechst 33342 was from Beyotime® Biotechnology Co. Ltd (Nantong, China). A near-infrared dye: 1,10-Dioctadecyl-3,3,30,30-tetramethylindo-tricarbocyanine Iodide (DiR), was from Biotium (Invitrogen, USA). DyLight® 488-labeled tomato lectin (Lycopersicon esculentum) came from Vector (USA). CD31 goat polyclonal primary antibody was from R&D (USA). BK goat polyconol primary antibody was from Santa Cruz Biotechnology (USA). FITC-labeled Affinipure donkey anti-goat secondary antibody and Cy™ 3-conjugated Affinipure donkey anti-goat secondary antibody were both obtained from Jackson (USA). Plastic cell culture dishes were purchased from the Corning Incorporation (USA). Foetal bovine serum (FBS), cell culture medium Dulbecco's modified Eagle's Medium (DMEM), trypsin-EDTA (0.25%) and penicillin-streptomycin were all from Gibco (CA). Deionised water was purified using a Millipore Simplicity System (Millipore, Bedford, MA) and was used throughout the study. All other reagents and chemicals were of analytical grade and were from Sinopharm Chemical Reagents (Shanghai, China).

Cells and animals

Human U87 glioma cells were obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China). Cells were cultured in DMEM containing 10% (v/v) FBS, 100 U/ml penicillin and 100 mg/ml streptomycin. Male BALB/c nude mice (weighing 20 ± 2 g) were from the Shanghai Slac Lab Animal Ltd. (Shanghai, China) and all animal experiments were performed in accordance with protocols stated by the ethics committee of Fudan University. U87 tumor xenografts bearing mice models were developed and the size of xenografts was measured as previously reported [14].

Tumor perfusion investigations

The effect of captopril on tumor perfusion was investigated by LDI and lectin labeling experiments. Mice bearing U87 tumor xenografts of a diameter of approximately 8 mm were injected i.p. with captopril at a dose of 100 mg/kg or an equal volume of saline and then subjected to a Periscan PIM 3 LDI system (Perimed AB, Stockholm, Sweden) for tumor blood perfusion detection at different time points (2, 8 and 24 h post drug injection). The associated scanning parameters were set and the data were collected and analyzed according to previous reports [31]. The specific method was introduced in Supporting information. In addition, lectin labeling experiment was also performed as previously reported [10,14].

SEM of tumor vessels

Mouse models with U87 tumor xenografts were injected i.p. with captopril at a dose of 100 mg/kg or an equal volume of saline, as described above. Two hours later, tumor xenografts were obtained, quickly cut into small particles about 1 mm^3, and

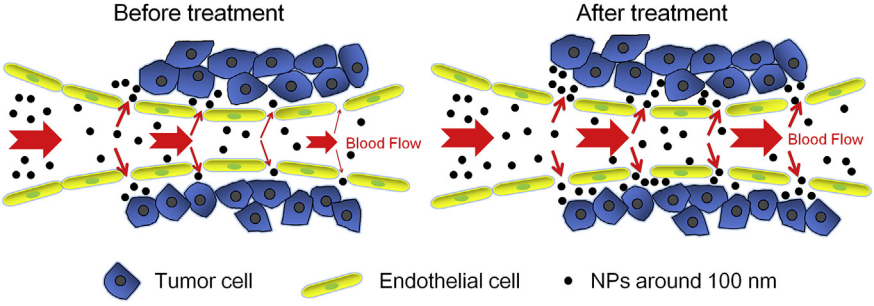

Fig. 1. Schematic graph of tumor perfusion, endothelial gaps and 100-nm NPs delivery to tumors with rich vasculature before and after captopril treatment. Before treatment, tumor perfusion was limited and endothelial gaps were smaller. These parameters worked together to compromise 100-nm NPs delivery to tumor tissues. In contrast, captopril treatment improved tumor perfusion and enlarged the endothelial gaps, consequently enhancing 100-nm NPs delivery to tumor tissues.