Acta Biomaterialia 58 (2017) 386–398

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Highly efficient delivery of potent anticancer iminoquinone derivative by multilayer hydrogel cubes

Bing Xue [a,1], Wei Wang [b,c,1], Jiang-Jiang Qin [b], Bhavitavya Nijampatnam [a], Srinivasan Murugesan [a], Veronika Kozlovskaya [a], Ruiwen Zhang [b,c,*], Sadanandan E. Velu [a,e,*], Eugenia Kharlampieva [a,d,*]

[a] Department of Chemistry, University of Alabama at Birmingham, Birmingham, AL 35294, United States
[b] Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, TX 79106, United States
[c] Cancer Biology Center, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, TX 79106, United States
[d] Center of Nanoscale Materials and Biointegration, University of Alabama at Birmingham, Birmingham, AL 35294, United States
[e] Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL 35294-3300, United States

ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form 15 May 2017
Accepted 2 June 2017
Available online 3 June 2017

Keywords:
Cubical hydrogel
Multilayer hydrogel
Intracellular degradable
Poly(methacrylic acid)
Hydrophobic drug
BA-TPQ
Hepatocellular carcinoma

ABSTRACT

We report a novel delivery platform for a highly potent anticancer drug, 7-(benzylamino)-3,4-dihydro-pyrrolo[4,3,2-de]quinolin-8(1H)-one (BA-TPQ), using pH- and redox-sensitive poly(methacrylic acid) (PMAA) hydrogel cubes of micrometer size as the encapsulating matrix. The hydrogels are obtained upon cross-linking PMAA with cystamine in PMAA/poly(N-vinylpyrrolidone) multilayers assembled within mesoporous sacrificial templates. The BA-TPQ-loaded hydrogels maintain their cubical shape and pH-sensitivity after lyophilization, which is advantageous for long-term storage. Conversely, the particles degrade *in vitro* in the presence of glutathione (5 mM) providing 80% drug release within 24 h. Encapsulating BA-TPQ into hydrogels significantly increases its transport via Caco-2 cell monolayers used as a model for oral delivery where the apparent permeability of BA-TPQ-hydrogel cubes was ~2-fold higher than that of BA-TPQ. BA-TPQ-hydrogel cubes exhibit better anticancer activity against HepG2 (IC_{50} = 0.52 μg/mL) and Huh7 (IC_{50} = 0.29 μg/mL) hepatoma cells with a 40% decrease in the IC_{50} compared to the non-encapsulated drug. Remarkably, non-malignant liver cells have a lower sensitivity to BA-TPQ-hydrogel cubes with 2-fold increased IC_{50} values compared to those of cancer cells. In addition, encapsulating BA-TPQ in the hydrogels amplifies the potency of the drug via down-regulation of MDM2 oncogenic protein and upregulation of p53 (a tumor suppressor) and p21 (cell proliferation suppressor) expression in HepG2 liver cancer cells. Moreover, enhanced inhibition of MDM2 protein expression by BA-TPQ-hydrogel cubes is independent of p53 status in Huh7 cells. This drug delivery platform of non-spherical shape provides a facile method for encapsulation of hydrophobic drugs and can facilitate the enhanced efficacy of BA-TPQ for liver cancer therapy.

Statement of Significance

Many potent anticancer drugs are hydrophobic and lack tumor selectivity, which limits their application in cancer therapy. Although cubical hydrogels of poly(methacrylic acid) exhibit excellent biocompatibility and versatility, they have not been investigated for hydrophobic drug delivery due to poor mechanical stability and incompatibility between hydrophobic drugs and a hydrophilic hydrogel network. In this study, we provide a facile method to prepare a multilayer hydrogel-based platform with controlled nanostructure, cubical shape and redox-responsiveness for delivery of highly potent anticancer therapeutics, hydrophobic BA-TPQ. The BA-TPQ-hydrogel cubes have exceptional structural stability upon lyophilization which is advantageous for a long-term storage. The greatly enhanced trans-epithelial permeability and amplified anti-tumor activity of BA-TPQ are achieved by encapsulation in these hydrogel cubes. Furthermore, the anticancer BA-TPQ-hydrogel platform retains the selective activity of BA-TPQ to hepatocellular carcinoma cells. Overall, the produced BA-TPQ-hydrogel cubes demonstrate a high potential for clinical liver cancer therapy.

* Corresponding authors at: Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, 1300 Coulter Street, Amarillo, TX, 79106, USA (R. Zhang). Department of Chemistry, University of Alabama at Birmingham, 901 14th Street South, CHEM280, Birmingham, AL 35294, USA (S.E. Velu). Department of Chemistry, University of Alabama at Birmingham, 901 14th Street South, CHEM291, Birmingham, AL 35294, USA (E. Kharlampieva).
E-mail addresses: ruiwen.zhang@ttuhsc.edu (R. Zhang), svelu@uab.edu (S.E. Velu), ekharlam@uab.edu (E. Kharlampieva).
[1] The authors equally contributed to this work.

http://dx.doi.org/10.1016/j.actbio.2017.06.004
1742-7061/

1. Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer death worldwide with high morbidity and mortality [1,2]. Current therapies have been shown to delay the development and progression of HCC to a certain extent: sorafenib, the predominant drug used for HCC treatment, can prolong the median survival of HCC patients by three months [3]. However, there is an urgent need to develop novel curative compounds to improve the therapeutic outcomes for patients with advanced HCC. An iminoquinone derivative, 7-(benzylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1H)-one (BA-TPQ), the most potent among the analogs of natural makaluvamine compounds, exerts anticancer activity against various cancer cells (e.g. breast cancer and prostate cancer) through multiple mechanisms including the suppression of the MDM2 oncoprotein, modulation of ZAK-MKK4-JNK-TGFβ signaling cascade, and the induction of endoplasmic reticulum stress [4–8]. Importantly, the BA-TPQ inhibition of tumor growth through down-regulation of MDM2 expression is independent of p53 status, demonstrating a broader range of anti-tumor effect even for the p53 mutant cancer cell lines [7]. However, despite its high potency against a variety of human cancer cell lines including breast and prostate tumors [8,9], the application of BA-TPQ has been limited by its poor solubility and low bioavailability. In addition, undesirable toxicity was observed in mice after administering BA-TPQ (a formulation of dispersed BA-TPQ in a mixture of PEG400/ethanol/0.9% saline) [8,9]. Recent investigations identifying the potential sites of accumulation to elucidate the observed BA-TPQ toxicity have revealed a high accumulation of BA-TPQ in the lungs, kidneys, and spleen of the mice [10,11]. Thus, an active drug delivery system for BA-TPQ is needed to improve anticancer efficacy and minimize side effects.

To surmount the limitations of unshielded therapeutic agents including low water-solubility, non-specific biodistribution, poor membrane permeability, multi-drug resistance, and toxicity towards non-malignant cells including those of bone marrow and the digestive tract [12–15], a variety of drug delivery platforms have been explored to improve the safety and efficiency of anticancer drugs [16–19]. Amphiphilic assemblies, such as micelles, polymersomes, and liposomes are widely used for hydrophobic drug delivery relying on physical entrapment of therapeutics within the hydrophobic polymer segments. However, their applications have been limited due to their potential structural instability *in vivo* where rapid vehicle disassembly may occur below the critical micelle concentration in the bloodstream [20].

Hydrogel particles have captivated the attention of researchers owing to their appealing physico-chemical properties and excellent versatility [21]. Their hallmark characteristics include large surface areas for functionalization, highly porous interior networks for drug loading, and easy tailoring of their geometry and surface morphology [22–24]. In contrast to organic or inorganic nanoparticles, hydrogels are soft materials which have elastic moduli ranging from 0.1 kPa to 100 kPa mimicking the elasticities of living cells and tissues and can regulate their bioactivities *in vitro* and *in vivo* [25–27]. Unlike self-assembled polymer structures such as micelles and liposomes, hydrogels possess exceptional mechanical stability because of their cross-linked structure and can undergo dramatic dimensional changes in response to stimuli in surrounding media causing on-demand drug release [28,29]. In addition to studies focusing on injectable drug delivery systems, hydrogel particles have been explored as potential carriers for oral delivery because of their excellent biocompatibility and diverse variety of material compositions [21,30].

Unlike the commonly used emulsion polymerization and precipitation polymerization methods for the synthesis of polymeric drug carriers, layer-by-layer (LbL) assembly of polymers at surfaces allows nanoscale control over the composition, chemistry, material thickness, and mechanical and stimuli-responsive properties of the resultant polymeric particles [31–33]. The LbL technique benefits from a vast selection of materials, as the interactions holding the multilayers together range from ionic-pairing and H-bonding to covalent and/or hydrophobic interactions [34,35]. The shape and size of LbL hydrogels are controlled by the respective dimensions of the colloidal templates which are dissolved after deposition to yield the polymeric networks [36]. In the case of non-porous templates, LbL assembly leads to hydrogel capsules with an interior cavity and nanothin walls, while alternating deposition of polymers inside porous templates results in nano-porous particles of interconnected macromolecular networks [37–40]. In the spirit of this concept we have recently demonstrated the synthesis of temperature- and pH-responsive nano-porous hydrogel microcapsules [41–43] and microparticles [37,38] of cubical shape. The cubic poly(methacrylic acid) (PMAA) hydrogel microparticles in this study were produced by sequential LbL infiltration of PMAA and poly(N-vinylpyrrolidone) (PVPON) inside 2 μm porous cubic templates [37,38]. After the sequential infiltration of (PMAA/PVPON) bilayers, the on-template assembly was cross-linked with ethylenediamine or cystamine using carbodiimide-assisted coupling.

While it is known that size, surface charge, and hydrogel rigidity control the cellular interactions of hydrogel particles, the shape of the hydrogel particle has also been found to have a strong impact on cellular interactions including intravascular and transvascular transport, the rate and percentage of cellular internalization, and biodistribution [44]. For instance, the internalization rate of PMAA multilayer hydrogel microcapsules by HeLa cancer cells decreased as the capsule aspect ratio increased when cylindrical shapes were used instead of spherical ones [45]. In contrast to spherical capsules, the cubical shape of soft capsules has been shown to be crucial for favorable interactions with endothelial HMVEC and 4T1, MDA-MB-231, and SUM159 breast cancer cells [46]. The cubical capsules exhibited highly increased internalization in the breast cancer cell lines, indicating the importance of their shape in the process of cellular uptake.

Although various drug delivery systems have been applied to deliver poorly water-soluble cancer curative agents (e.g. paclitaxel, 5-fluorouracil and curcumin), the vehicular transport of BA-TPQ by non-spherical polymeric carriers has yet to be reported. Stimuli-responsive microgels with tailored shape for hydrophobic drug delivery have also been underexplored, which is mainly attributed to: (1) The highly water-favoring nature of the network which limits the interaction between microgel and hydrophobic molecules; (2) The synthetic challenges associated with fabricating and maintaining the 3-dimensional structure of shaped submicron-sized hydrogels without compromising stimuli-responsiveness. In previous works, the applications of pH [37] and dual pH/redox [38] PMAA hydrogel particles of cubical shape for encapsulation of the hydrophilic drug doxorubicin have been demonstrated. Nevertheless, the capability of this type of hydrogel for the transport of hydrophobic remedies has not been investigated. In addition, the effect of utilizing hydrogel particles as drug carriers on the therapeutic activity of BA-TPQ against tumor cells is still unknown.

In this work, a facile method has been developed to encapsulate BA-TPQ within the network of cubic PMAA hydrogel microparticles for liver cancer treatment. The polymer network in the multilayer hydrogels is "packed" throughout the volume of the cubes, providing an enormous surface area and structural stability which facilitates encapsulation of the hydrophobic BA-TPQ. The structural stability, pH- and redox-responsiveness, and their effects on loading and release of BA-TPQ has also been investigated. The