Journal of Controlled Release 182 (2014) 13–21

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Delivering wasp venom for cancer therapy

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ARTICLE INFO

Article history:
Received 10 February 2014
Accepted 3 March 2014
Available online 12 March 2014

Keywords:
Pro-cytotoxic systems
Cytolytic peptides
Polyglutamic acid
Targeting peptides
Overexpressed tumor proteases
HER2 + breast cancer

ABSTRACT

Cytolytic peptides with potential therapeutic properties have appeared during the last three decades. However, the use of these natural weapons is relatively narrow due to their non-specific cytolytic activity as well as their rapid degradation and excretion when injected in blood. In order to rescue the use of these lytic peptides, we have designed pro-cytotoxic systems based on cytotoxic peptides conjugated to poly(L-glutamic acid) PGA polymer through specific cleavage sequences that are sensitive over-expressed tumor proteases, such as the metalloproteinase-2 (MMP-2) or cathepsin B. The potent cytotoxic peptide tested here, Mitoparan, is inactive when conjugated to the polymer and then become active again once released through the tumor proteases. Furthermore, this pro-cytotoxic system was decorated by a particular targeting peptide which binds to HER2 receptors over-expressed in some types of breast tumor cells, thereby increasing the selective release of cytolytic peptides inside tumor cell with exquisite spatiotemporal control. In this way, the system would improve the maximum tolerated dose and the pharmacokinetic parameters of cytotoxic peptides in vivo.

1. Introduction

The use of venoms in cancer therapy continues to be a challenge. These natural weapons have been widely studied for the treatment of several immune-related diseases, and have recently entered preclinical phases for cancer treatment [1]. However, the high toxicity of these potential therapeutic peptides caused by non-specific lytic activity and their rapid degradation in blood make them of limited use in cancer therapy. These peptides are between 10 and 50 residues in length, and they show amphipathic properties. They have a propensity to interact with membranes, oligomerizing on the cell surface so as to form transient pores, thus causing cell death. Since free cytolytic peptides are not able to elicit a therapeutic benefit at a safe dose, they have to be targeted and delivered as pro-drugs. Previous studies report on how different types of cytolytic peptides have been successfully conjugated to proteins through tumor-specific proteases, such as metalloproteinase-2 (MMP-2) [2,3]. In addition, cytotoxic peptides have been carried by lipidic systems, as in Yamada's work [4], where the peptide mastoparan was transported inside a transferrin-modified liposome. The most recent case is the 26-mer cytotoxic peptide melittin, derived from bee venom, which has been successfully targeted and delivered via fluorocarbon nanocarriers to tumor models in vivo [5,6]. Further improvements in order to increase the efficacy of the systems are needed. Alternative types of carriers, such polymers, with suitable features could improve the efficacy of delivery cytolytic peptides, overcoming some drawbacks related to low activity, elaborate complexes and non-scalable production. In fact, an increasing number of polymer therapeutics have started to move from basic science into clinical trials and, even into clinical practice [7]. Not only should the polymer in the drug delivery system be innocuous, hydrophilic, non-immunogenic and biodegradable, but also should it have a long-circulating behavior and enhanced accumulation at the tumor site. In particular, the well-characterized poly(L-glutamic acid) (PGA), which has an attractive safety profile, has been widely used with many kinds of cargo, such as low MW drugs [8] and, even, peptoids [9].

In this study, we present a peptide-polymer design strategy to obtain pro-cytotoxic systems based on lytic peptides conjugated to PGA polymer through specific cleavage sites that are sensitive over-expressed tumor proteases, such as MMP-2 or cathepsin B (Fig. 1). The potent cytotoxic peptides are inactive when conjugated to the polymer, but once released through the tumor proteases, they recover their activity. This strategy is thought to prevent the side effects that occur in vivo. Furthermore, this pro-cytotoxic carrier was decorated with peptides able to specifically target tumor cells. In this way, the system would improve the maximum tolerated dose and the pharmacokinetic parameters of cytotoxic peptides in vivo.

2. Materials and methods

2.1. Materials

Fmoc-Nα-protected amino acids and poly(L-glutamic acid) (Npt-PGA(100)Na) were obtained from IRIS Biotech GmbH (Marktredwitz,

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http://dx.doi.org/10.1016/j.jconrel.2014.03.005
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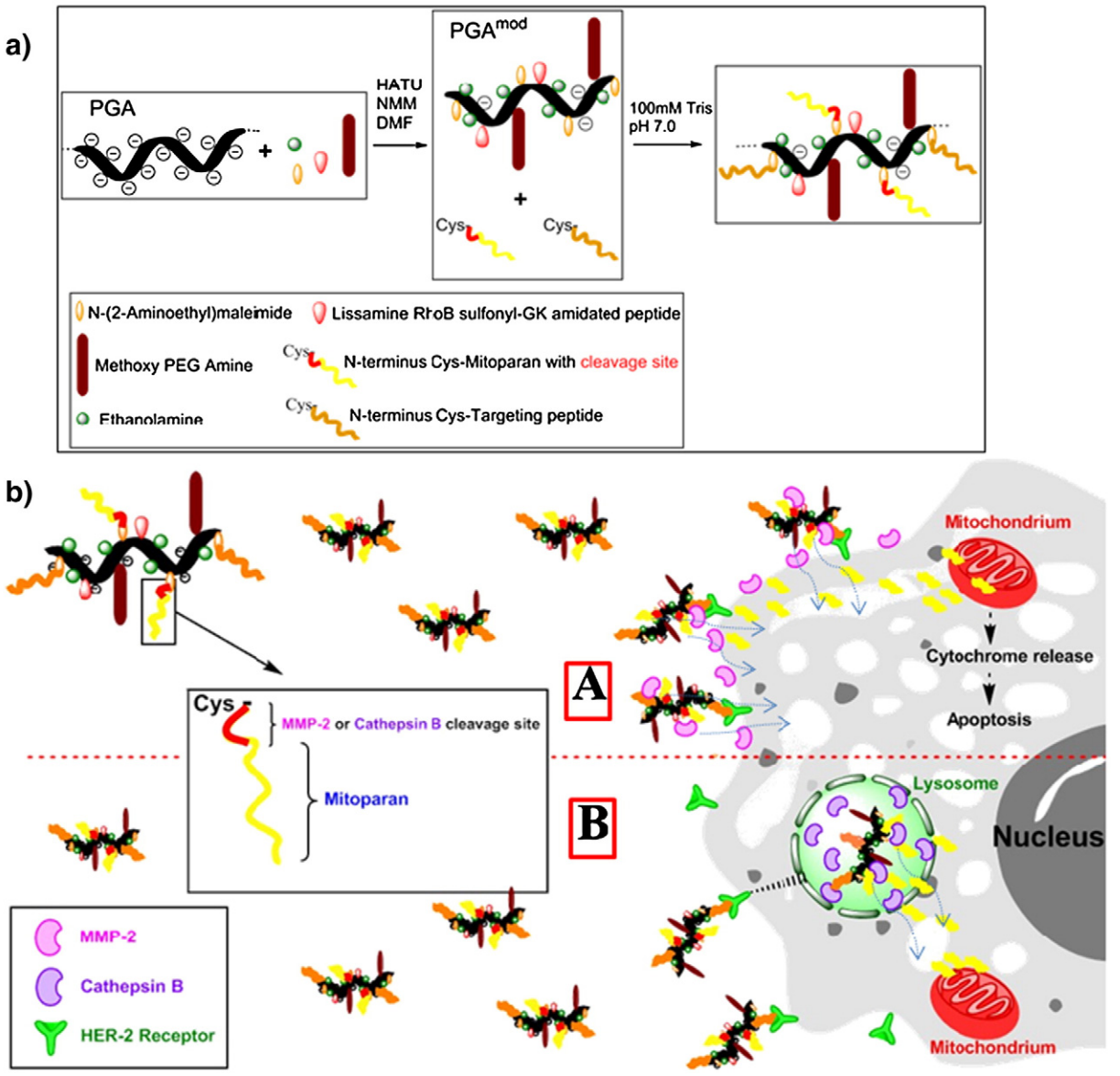

Fig. 1. a) Synthetic strategy of the targeted pro-cytotoxic conjugate based on modified PGA polymer (PGA^{mod}). PGA decoration with different reagents is used to prepare a soluble scaffold onto which the targeting and pro-apoptotic peptides (Mitoparan) are attached. b) Schematic illustration showing the interaction of targeted pro-cytotoxic conjugates with HER2 positive tumor cells, which overexpress MMP-2 on the plasmatic membrane (A) or cathepsin B inside lysosomes (B). These two enzymes will promote the release of Mitoparan, cutting through specific cleavage sequences, transforming this pro-drug system into a toxic drug.

Germany). The Fmoc-Rink-Amide AM polystyrene resin was purchased from CBL-PATRAS (Patras, Greece). Coupling reagents: 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) was from Albatros Chem, Inc. (Montreal, Canada). Trifluoroacetic acid (TFA) was purchased from Scharlab S.L. (Barcelona, Spain). Piperidine, dimethylformamide (DMF), dichloromethane (DCM) and acetonitrile (MeCN) were from SDS (Peypin, France). N,N-Diisopropylethylamine (DIEA) was obtained from Merck (Darmstadt, Germany). Tri-isopropylsilane (TIS) was from Fluka (Buchs, Switzerland). Lissamine rhodamine B sulfonyl chloride (Sulrhodamine B) was obtained from Acros (Somerville, NJ). Methoxy PEG Amine, HCl Salt, MW 5000 was from JenKem Technology USA Inc. LysoTracker® Green DND-26 and Red DND-99 were supplied by Invitrogen (Carlsbad, CA). Cathepsin B from human liver, Sephadex® G-25, 5(6)-carboxyfluorescein (CF), tetrakis(triphenylphosphine)palladium(0), ($Pd(PPh_3)_4$), phenylsilane ($PhSiH_3$) and ethanolamine were obtained from Sigma-Aldrich (St. Louis, USA). Recombinant Human Matrix Metalloproteinase 2 (MMP-2) was purchase from Sina Biological Inc. (Beijing, China).

2.2. Peptide synthesis

Peptides were synthesized by solid phase synthesis using the 9-fluorenylmethoxycarbonyl/tert-butyl (Fmoc/tBu) strategy. Fmoc-Rink-Amide AM polystyrene resin, Nα-Fmoc-protected amino acids (3 eq)/TBTU(3 eq.), and DIEA(6 eq.) were used. The Fmoc protecting group was cleaved by treatment with a solution of 20% piperidine in DMF. For labeling with 5(6)-carboxyfluorescein (CF) of the dye-peptides 2, 3, and 9, firstly, Nα-Fmoc-Lys(Alloc)-OH was incorporated at the desired position. For the Alloc group removal, the resin was treated with $Pd(PPh_3)_4$ and $PhSiH_3$ (3 × 15 min) and then washed with DMF. Afterwards, 2 treatments of 30 min with a solution of sodium N,N′ diethyldithiocarbamate in DMF were performed. Finally, the CF was incorporated as it was a Nα-Fmoc-protected amino acid. Peptides were cleaved from the resin by treatment with 95% TFA, 2.5% TIS, 2.5% water for 3 h and detected by analytical RP-HPLC Waters 996 photodiode array detector equipped with the Waters 2695 separation module, the Symmetry column (C 18, 5 mm, 4.6P150 mm) and the Millennium