European Journal of Medicinal Chemistry 47 (2012) 560–572

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Dendrimer-based multivalent methotrexates as dual acting nanoconjugates for cancer cell targeting

Ming-Hsin Li [a,b], Seok Ki Choi [a,*], Thommey P. Thomas [a], Ankur Desai [a], Kyung-Hoon Lee [a,c], Alina Kotlyar [a], Mark M. Banaszak Holl [a,b,c,d], James R. Baker Jr. [a,b,**]

[a] Michigan Nanotechnology Institute for Medicine and Biological Sciences, and Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109, USA
[b] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA
[c] Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA
[d] Department of Macromolecular Science and Engineering, University of Michigan, Ann Arbor, MI 48109, USA

ARTICLE INFO

Article history:
Received 8 September 2011
Received in revised form
8 November 2011
Accepted 15 November 2011
Available online 23 November 2011

Keywords:
Folate receptor
Methotrexate
Poly(amidoamine) dendrimer
Targeted drug delivery
Multivalent binding
Dual activity

ABSTRACT

Cancer-targeting drug delivery can be based on the rational design of a therapeutic platform. This approach is typically achieved by the functionalization of a nanoparticle with two distinct types of molecules, a targeting ligand specific for a cancer cell, and a cytotoxic molecule to kill the cell. The present study aims to evaluate the validity of an alternative simplified approach in the design of cancer-targeting nanotherapeutics: conjugating a single type of molecule with dual activities to nanoparticles, instead of coupling a pair of orthogonal molecules. Herein we investigate whether this strategy can be validated by its application to methotrexate, a dual-acting small molecule that shows cytotoxicity because of its potent inhibitory activity against dihydrofolate reductase and that binds folic acid receptor, a tumor biomarker frequently upregulated on the cancer cell surface. This article describes a series of dendrimer conjugates derived from a generation 5 polyamidoamine (G5 PAMAM) presenting a multivalent array of methotrexate and also demonstrates their dual biological activities by surface plasmon resonance spectroscopy, a cell-free enzyme assay, and cell-based experiments with KB cancer cells.

1. Introduction

Nanotechnology is uniquely suited for providing multifunctional platforms for targeted delivery in several life-threatening diseases, including cancers and inflammatory diseases [1–6]. This study aims to investigate a novel and simplified delivery strategy based on functionalization of a nanoplatform with a dual-acting small molecule, in lieu of two single-acting molecules, that serves as both a targeting ligand and an anticancer therapeutic. In this communication, we demonstrate the validity and effectiveness of this simplified strategy by designing multivalent NPs presenting methotrexate on the periphery of a generation 5 polyamidoamine (G5 PAMAM) dendrimer.

Applications of multifunctional NPs in anticancer therapeutic delivery have been well demonstrated by use of targeting ligands specific for a cell surface molecule overexpressed in cancer cells, such as folic acid receptor (FAR) [7–9], riboflavin receptor [10,11], $\alpha_v\beta_3$ integrin [12–14], prostate-specific membrane antigen [15], Her2 [16], transferrin receptor [17], and epidermal growth factor receptor [16,18,19]. The biological process for the effective uptake of such NPs requires multiple-ligand molecules attached to the NP surface. It begins with specific adhesion of an NP to the targeted cell surface in a mechanism that is characterized by multivalent interactions occurring collectively at the interface of multiple receptor-ligand pairs [12,20]. Such a multivalent mechanism is considered as highly important during the receptor-mediated endocytosis because it constitutes the basis for tight NP-cell adhesion and conformal contacts created during the formation of coated pits [21–24]. Therefore, in a rational design for targeted NPs, each NP is covalently conjugated with multiple copies of a targeting ligand on its periphery in order to achieve the multivalent effects, and each is further functionalized to carry therapeutic or imaging molecules as the payloads for cellular delivery [1,25–27].

Despite the rational basis of the NP design and successful proof of concept studies demonstrated already, several challenging issues face the development of cancer-targeting therapeutic NPs. They are

Abbreviations: FA, folic acid; FAR, folic acid receptor; MTX, methotrexate; PAMAM, polyamidoamine; SPR, surface plasmon resonance; DHFR, dihydrofolate reductase.

* Corresponding author. Tel.: +1 734 615 0618; fax: +1 734 615 0621.
** Corresponding author. Michigan Nanotechnology Institute for Medicine and Biological Sciences, and Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109, USA. Tel.: +1 734 647 2777; fax: +1 734 615 2506.
E-mail addresses: skchoi@umich.edu (S.K. Choi), jbakerjr@umich.edu (J.R. Baker).

0223-5234/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.11.027

attributable simply to the complexity of the NP structure and the lack of methods to control the distribution of the particle size, ligand density, and drug loads [27–31]. Currently, there are only a few specialized methods demonstrated for the precise engineering and ligand functionalization of NPs such as PAMAM dendrimer [28,30,32], polymer [33], or gold [34,35]. To ease the complexity of the fabrication of targeted NP therapeutics, we have explored new design strategies. Here we evaluate the feasibility of using a dual-acting small molecule that can: i) function as a ligand for a cancer-specific receptor, and ii) induce cytotoxicity following cellular internalization. Because this approach is based on using a single type of small molecule for both targeting and functional activity, the precision by which these functionalized nanoparticles can be synthesized is higher than what can be obtained using the conventional two small molecule approach.

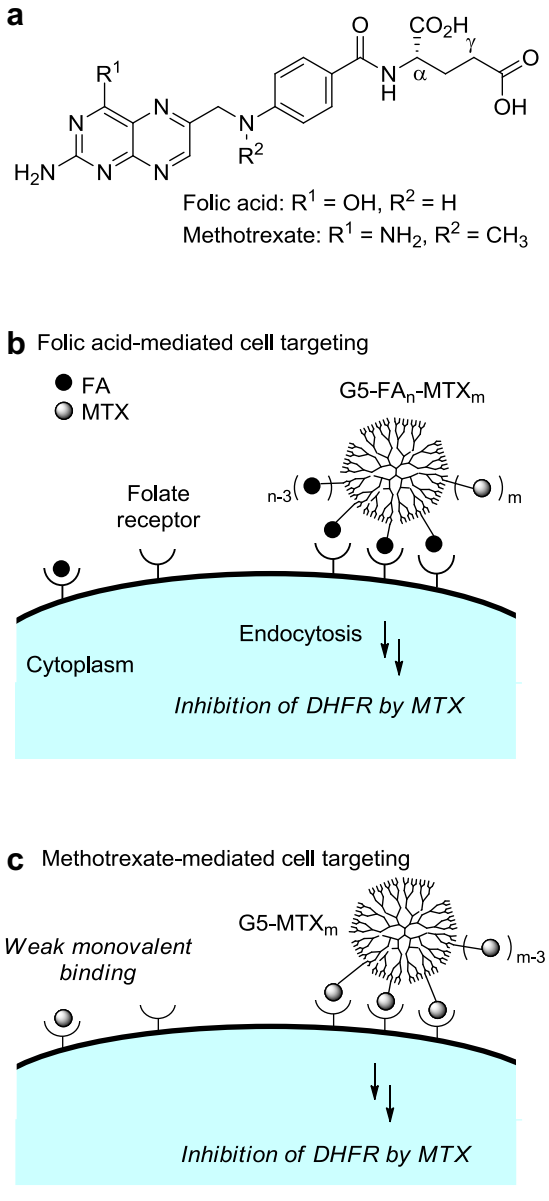


Fig. 1. (a) Structure of folic acid (FA), and methotrexate (MTX); (b) Schematic for the folic acid receptor (FAR)-mediated cancer cell targeting by a dendrimer nanoconjugate ($G5\text{-}FA_n\text{-}MTX_m$) presenting FA as a targeting ligand and carrying MTX as a cytotoxic drug; (c) Schematic for the proposed folic acid receptor (FAR)-mediated cancer cell targeting by a dendrimer nanoconjugate ($G5\text{-}MTX_n$) presenting methotrexate (MTX) as a dual-acting molecule for targeting and the cytotoxic activity. The figure is not drawn to scale.

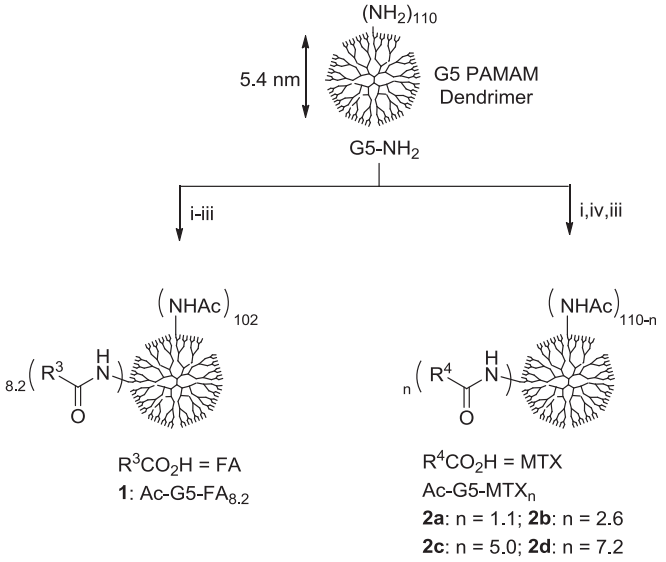
In our search for candidate molecules that could both target and function as a therapeutic, we were interested in methotrexate (MTX, Fig. 1), which has been used as an anticancer drug [5,6]. This therapeutic molecule functions primarily by inhibiting the metabolic enzymes human dihydrofolate reductase (DHFR), an enzyme localized in the cytoplasm (K_i = 1.2 nM) [36]. While the cellular uptake of MTX is mediated by reduced folate carrier proteins [37], MTX is also able to bind FAR because of its high structural homology to FA, though at a lower affinity constant (K_D = ~20–100 nM vs. K_D (FA) = ~1 nM to kidney FAR) [38–40].

Despite the lower FAR affinity of MTX, we hypothesize that MTX could still function as a targeting ligand if multiple copies per nanoparticle were utilized to form a multivalent nanoparticle [21–24]. Recently, we designed a class of synthetic multivalent nanoparticles, each composed of multiple FA ligands conjugated to a G5 PAMAM dendrimer scaffold, and demonstrated by surface plasmon resonance (SPR) spectroscopy that nanoparticles functionalized with more than one FA bound more tightly to a bovine folate binding protein (FBP)-coated surface than free FA [20,41]. Based on this observation, we aimed to determine whether a multivalent MTX-based dendrimer is still effective for FAR targeting. A recent theoretical analysis suggests that multivalent cooperativity can be kinetically limited if the binding of an individual receptor-ligand pair is too tight [42]. It further suggests that the targeting specificity for a particular cell type can be enhanced by making the affinity of each individual receptor-ligand pair weaker. In this study, we describe the synthesis of multivalent MTX conjugates and provide evidence, based on SPR and *in vitro* studies, that these conjugates possess the dual activity necessary for serving as an effective cancer therapeutic.

2. Results and discussion

2.1. Surface plasmon resonance (SPR) spectroscopy of multivalent MTX conjugates

We studied the effect of multivalency on the interaction between FBP present on the surface and a dendrimer functionalized with MTX molecules by using SPR spectroscopy. As a bioanalytical



Scheme 1. A representative synthetic scheme for G5 PAMAM dendrimers presenting either folic acid (FA) or methotrexate (MTX) molecule in multiple copies. (i) Ac_2O, MeOH, rt; (ii) folic acid, EDC, DMSO, DMF, rt, 24 h; (iii) Ac_2O, DMSO, rt; (iv) variable amounts of methotrexate, EDC, DMF, DMSO, rt, 24 h.