Biomaterials 77 (2016) 243–254

Contents lists available at ScienceDirect

Biomaterials

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

Hitchhiking nanoparticles: Reversible coupling of lipid-based nanoparticles to cytotoxic T lymphocytes

Laura Wayteck [a], Heleen Dewitte [a], Lynn De Backer [a], Karine Breckpot [b], Jo Demeester [a], Stefaan C. De Smedt [a, *, 1], Koen Raemdonck [a, 1]

[a] Laboratory of General Biochemistry and Physical Pharmacy, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium
[b] Laboratory of Molecular and Cellular Therapy, Department of Biomedical Sciences, Vrije Universiteit Brussel, Laarbeeklaan 103/E, 1090 Brussels, Belgium

ARTICLE INFO

Article history:
Received 4 November 2015
Accepted 6 November 2015
Available online 10 November 2015

Keywords:
Cell-mediated drug delivery
Tumor targeting
Cytotoxic T lymphocytes
Nanoparticles
Liposomes

ABSTRACT

Following intravenous injection of anti-cancer nanomedicines, many barriers need to be overcome en route to the tumor. Cell-mediated delivery of nanoparticles (NPs) is promising in terms of overcoming several of these barriers based on the tumoritropic migratory properties of particular cell types. This guided transport aims to enhance the NP accumulation in the tumor and moreover enhance the infiltration of regions that are typically inaccessible for free NPs. Within this study, cytotoxic $CD8^+$ T cells were selected as carriers based on both their ability to migrate to the tumor and their intrinsic cytolytic activity against tumor cells. Many anti-cancer nanomedicines require tumor cell internalization to mediate cytosolic drug delivery and enhance the anti-cancer effect. This proof-of-concept therefore reports on the reversible attachment of liposomes to the surface of cytotoxic T lymphocytes via a reduction sensitive coupling. The activation status of the T cells and the liposome composition are shown to strongly influence the loading efficiency. Loading the cells with liposomes does not compromise T cell functionalities like proliferation and cytolytic function. Additionally, the triggered liposome release is demonstrated upon the addition of glutathione. Based on this optimization using liposomes as model NPs, a small interfering RNA (siRNA)-loaded NP was developed that can be coupled to the surface of $CD8^+$ T cells.

1. Introduction

Nanoparticles (NPs) have been widely investigated for the delivery of anti-cancer drugs to tumor tissues [1–3]. Although a lot of research has been performed, to date, only few nanomedicines are in clinical use for the treatment of cancer [4,5]. One of the key challenges is inadequate targeted delivery of drug-loaded NPs to the tumor tissue following systemic administration. A critical barrier remains the vascular endothelium which controls the extravasation of NPs from the bloodstream into the target tissue [6,7]. Because NP extravasation is dependent on the size of fenestrae, tumor tissue may favor this delivery route by its leaky blood vasculature. This increased extravasation in combination with the poor lymphatic drainage found in tumors results in the accumulation of NPs in the tumor tissue and is also known as the enhanced permeation and retention (EPR) phenomenon [8]. Unfortunately, the widespread application of the EPR-based passive targeting of tumors is hampered by the heterogeneity in tumor vasculature both within a single tumor and between different tumor types [9].

Besides relying on passive targeting alone, nanomedicines are frequently modified with a targeting ligand that specifically recognizes a receptor that is present on or overexpressed by tumor cells. Several studies have shown that this active targeting strategy enhances the internalization in the target cells, albeit without increasing the accumulation in the tumor tissue after systemic delivery [10–12]. Furthermore, it was observed that active targeting can hinder the diffusion of NPs into the tumor tissue due to the binding-site barrier phenomenon in which particles mainly bind to perivascular cells [1].

* Corresponding author.
E-mail address: Stefaan.Desmedt@Ugent.be (S.C. De Smedt).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biomaterials.2015.11.016
0142-9612/

One alternative strategy that might overcome these targeting limitations is cell-mediated delivery [13,14]. Certain cell types have the intrinsic ability to cross the endothelial barrier and infiltrate the tumor tissue, which makes them attractive carriers for NP delivery [15]. Moreover, tumors contain hypoxic and necrotic regions that are typically not easily accessible for NPs, leading to incomplete tumor regression [16,17]. Monocytes and macrophages have already been investigated as carriers for the delivery of NPs in these low accessible regions [18–20]. Also T cells have the intrinsic ability to migrate to the tumor tissue. Moreover, cytotoxic $CD8^+$ T lymphocytes (CTLs) have the added advantage of exerting a cytotoxic effect on tumor cells upon their recognition and binding of tumor-associated antigens. By virtue of these specific traits, the adoptive transfer of T cells is a widely investigated cancer immunotherapy. Adoptive T cell therapy can be described as the transfer of *ex vivo* selected and expanded tumor-reactive T cells [21]. Although adoptive T cell therapy already showed impressive clinical outcomes, complete tumor regression was only observed in a restricted number of patients mainly due to the immunosuppressive tumor microenvironment and also the loss of tumor antigens to which the therapy was targeted [22,23]. Therefore, strategies combining conventional chemotherapies with cancer immunotherapy have gained interest in recent years [24].

The use of T cells as NP carrier cells has been assessed previously by Irvine and colleagues, who irreversible attached drug-loaded NPs to their surface and also by Vile and co-workers, who exploited T cells for the intratumoral delivery of therapeutic viral vectors [25–27]. Here, we propose a synergistic strategy based on CTLs that serve a dual purpose, i.e. induce a direct killing effect on tumor cells and enhance the delivery of drug-loaded NPs into tumor tissue, which may kill T cell-refractory tumor cells. To this end, we investigated the covalent attachment of NPs to the surface of CTLs. Moreover, we envisioned the triggered release of the cell surface-conjugated NPs in the tumor microenvironment which could allow their subsequent internalization by target tumor cells and hence a more efficient delivery of anti-cancer drugs. This concept is of particular interest for charged macromolecular therapeutics that are membrane-impermeable such as small interfering RNA (siRNA), which require NP-mediated cytosolic delivery in target cells. To date, a reversible coupling strategy of NPs to the surface of CTLs has not yet been investigated.

Given their outstanding promise and versatility as drug delivery systems, we used liposomes as model nanomedicines within this proof-of-concept [28]. First, it was assessed whether liposomes can be attached to the surface of CTLs based on disulfide bond formation between exofacial thiols present on the CTL plasma membrane and thiol-reactive liposomes. Next, we showed the triggered release of liposomes in the presence of glutathione as a reducing agent. Importantly, *in vitro* data indicated that the NP-load did not affect the T cell functionalities such as proliferation and cytotoxic effector functions.

It has been demonstrated that the adoptive transfer of less differentiated T cells is associated with superior persistence and anti-tumor responses compared to the more differentiated effector T cells [29,30]. On the other hand, the differentiation of naive T cells into $CD8^+$ effector T cells is required for their migratory and cytotoxic function. Since both activated and naive T lymphocytes are investigated for adoptive T cell therapy, we therefore evaluated the reversible coupling of liposomes as a function of their activation status [31].

Finally, building further on this optimized reversible coupling strategy, we demonstrated the coupling of lipid-enveloped nanogels, loaded with siRNA, to the surface of activated $CD8^+$ T cells.

2. Materials and methods

2.1. Preparation of liposomes and characterization

The liposomes were composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), egg phosphatidylglycerol (egg PG) or 18:1 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio)propionate] (PE-PDP), and the fluorescent 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine (DiD). DOPC and PE-PDP lipids were purchased from Avanti Polar Lipids (Alabaster, USA), PG lipids from Lipoid (Ludwigshafen, Germany), and DiD from Molecular probes (Invitrogen, Merelbeke, Belgium). The typical liposome composition comprised 50 wt% PE-PDP or PG, 3 wt % DiD, and 47 wt% DOPC. When varying the PE-PDP or PG content, the fraction of DOPC lipids was adjusted accordingly. To form the liposomes, a lipid mixture in chloroform was prepared and pipetted in a round-bottom glass flask, followed by evaporation of the chloroform solvent to form a thin lipid film. The dried lipids were rehydrated in phosphate buffered saline (PBS; Gibco-Invitrogen, Merelbeke, Belgium) at ambient temperature followed by an extrusion of the resultant multilamellar liposomes through a 200 nm polycarbonate filter (Whatman, Diegem, Belgium). Liposome size and surface charge were measured in HEPES buffer (20 mM, pH 7.4) at 25 °C by dynamic light scattering and zeta potential measurements, respectively, using a Zetasizer Nano ZS (Malvern, UK) equipped with Dispersion Technology Software (DTS).

2.2. Cell culture

$CD8^+$ T cells were isolated from the spleen of OT-I mice using a negative isolation $CD8^+$ kit (stem cell technologies, Grenoble, France). Cells were activated with anti-CD3/CD28 Dynabeads® (Gibco-Invitrogen) at a density of 2×10^6 cells/well and a bead-to-cell ratio of 1:1 in a 24-well plate. The cells were cultured in complete T cell culture medium containing advanced RPMI medium (Gibco-Invitrogen) supplemented with 10% fetal bovine serum (FBS; Hyclone, Thermo Scientific, MA, USA), 2 mM L-glutamine (Gibco-Invitrogen), 1% penicillin/streptomycin (Gibco-Invitrogen). According to the manufacturer's protocol 30 U ml^{-1} rIL-2 (Miltenyi Biotec, Leiden, The Netherlands) was added to the culture medium and restimulation was performed by adding new beads every 7 days.

The mouse thymoma cell lines EL4 and E.G7-OVA were purchased from the American Type Culture Collection (ATCC). The E.G7-OVA cell line is an EL4 cell line that was stably transfected with the plasmid pAc-neo-OVA, which carries a complete copy of chicken ovalbumine (OVA) mRNA. Both cell lines were cultivated in RPMI 1640 (Gibco-Invitrogen) supplemented with 10% FBS, 2 mM L-glutamine, and 1% penicillin/streptomycin.

2.3. Visualization of exofacial thiol groups

$CD8^+$ T cells were incubated for 15 min with 100 μg ml^{-1} Cy5-labeled maleimide (GE Healthcare Life Sciences, Diegem, Belgium) at room temperature. After washing the cells with PBS, the cells were fixed with 4% paraformaldehyde (Sigma–Aldrich, Bornem, Belgium). Confocal microscopy images were acquired using a Nikon C1si confocal laser scanning microscope (Nikon Benelux, Brussels, Belgium) equipped with a Plan Apo VC 60x 1.4 NA oil immersion objective lens (Nikon). The Cy5 dye was excited with a 636 nm diode laser (CVI Melles Griot, NM, USA).