Biomaterials 111 (2016) 116–123

Contents lists available at ScienceDirect

Biomaterials

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

Nanoparticles camouflaged in platelet membrane coating as an antibody decoy for the treatment of immune thrombocytopenia

Xiaoli Wei [a, b, 1], Jie Gao [a, b, 1], Ronnie H. Fang [a], Brian T. Luk [a], Ashley V. Kroll [a], Diana Dehaini [a], Jiarong Zhou [a], Hyeon Woo Kim [a], Weiwei Gao [a], Weiyue Lu [b], Liangfang Zhang [a, *]

[a] Department of NanoEngineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA
[b] Department of Pharmaceutics, School of Pharmacy, Fudan University, Key Laboratory of Smart Drug Delivery (Fudan University), Ministry of Education, Shanghai 201203, PR China

ARTICLE INFO

Article history:
Received 19 August 2016
Received in revised form
30 September 2016
Accepted 2 October 2016
Available online 5 October 2016

Keywords:
Autoimmune disease
Platelet membrane-coated nanoparticle
Biomimetic nanoparticle
Nanosponge
Antibody decoy

ABSTRACT

Immune thrombocytopenia purpura (ITP) is characterized by the production of pathological autoantibodies that cause reduction in platelet counts. The disease can have serious medical consequences, leading to uncontrolled bleeding that can be fatal. Current widely used therapies for the treatment of ITP are non-specific and can, at times, result in complications that are more burdensome than the disease itself. In the present study, the use of platelet membrane-coated nanoparticles (PNPs) as a platform for the specific clearance of anti-platelet antibodies is explored. The nanoparticles, whose outer layer displays the full complement of native platelet surface proteins, act as decoys that strongly bind pathological anti-platelet antibodies in order to minimize disease burden. Here, we study the antibody binding properties of PNPs and assess the ability of the nanoparticles to neutralize antibody activity both *in vitro* and *in vivo*. Ultimately, we leverage the neutralization capacity of PNPs to therapeutically treat a murine model of antibody-induced thrombocytopenia and demonstrate considerable efficacy as shown in a bleeding time assay. PNPs represent a promising platform for the specific treatment of antibody-mediated immune thrombocytopenia by acting as an alternative target for anti-platelet antibodies, thus preserving circulating platelets with the potential of leaving broader immune function intact.

1. Introduction

Platelets, also known as thrombocytes, are a blood component that is essential for maintaining hemostasis. One of their main functions is to stop bleeding via initiation and propagation of the coagulation cascade [1,2]. Platelet count is universally regarded as the key indicator of bleeding risk, and the normal range in healthy people sits between 150,000 and 450,000 platelets per microliter of blood. A count under the normal range, termed thrombocytopenia, can be due to either decreased platelet production or increased platelet destruction. Clinically, the disease can manifest itself as purpura, a delay in the normal process of clotting, and spontaneous or excessive bleeding. When platelet counts drop substantially lower than normal values, internal hemorrhaging can occur, a severe condition that can potentially be fatal [3].

Immune thrombocytopenia purpura (ITP), which is oftentimes also referred to as idiopathic thrombocytopenic purpura, is an immune-mediated hematological disorder characterized by low level of platelets and easy or excessive bleeding due to the presence of anti-platelet autoantibodies [4,5]. These pathological antibodies bind to specific antigens on the platelet surface, leading to sequestration and destruction by the reticuloendothelial system. The age-adjusted prevalence of ITP is estimated to be 9.5 per 100,000 persons in the United States [6]. While the condition may appear secondary to a known autoimmune condition or infection, oftentimes the underlying etiology is unclear [7–9]. Given this fact, chronic ITP is classically treated using nonspecific therapies such as corticosteroids. While capable of eliciting a rebound in platelet levels in many patients, such treatments are susceptible to relapse and can cause unwanted side effects [5,10]. For those that fail to respond to frontline treatments, invasive and irreversible splenectomy is a common intervention, but has the chance of

* Corresponding author.
E-mail address: zhang@ucsd.edu (L. Zhang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biomaterials.2016.10.003
0142-9612/

postoperative complications such as infection, bleeding, and hospitalization [11,12]. Other second- and third-line treatments include intravenous immunoglobulin (IVIg) [13], intravenous Rho immunoglobulin (RhIg) [14], rituximab (anti-CD20) [15], and thrombopoietin receptor agonists [16]. Most carry significant iatrogenic risk given their generally non-specific modes of action. With the probability of high side effects, treatment can ultimately be more burdensome than the original disease. With these considerations in mind, the development of a treatment modality that can specifically target the pathological moieties responsible for ITP is highly desirable.

Cell membrane-coated nanoparticles represent an increasingly popular platform for a variety of applications, including drug delivery [17], vaccination [18,19], and detoxification [20,21]. A significant factor behind their appeal is the ability to replicate the surface properties of different cell types faithfully on nanoparticle surfaces. Employing biological materials through a top-down coating approach bestows synthetic nanoparticles with native cell functionalities. For example, it has been shown that coating with red blood cell membrane actively modulates residence time in the bloodstream via the display of self-markers that are recognized by the immune system [22]. Functionalization with platelet membrane enables biomimetic targeting by taking advantage of the natural interactions between platelet surface markers and different targets, including damaged vasculature and pathogens [23,24]. Given the wide range of biological interactions that natural cell membranes participate in, the potential of cell membrane-coated nanoparticles extends far beyond traditional nanodelivery applications. One such area is biodetoxification where the membrane coating serves as an ideal substrate for interaction with biological toxins, enabling their neutralization and subsequent clearance. For example, red blood cell membrane-coated nanoparticles have previously been shown to bind and clear both bacterial toxins [20] as well as small molecule poisons [21].

Here, we demonstrated the use of platelet-derived membrane as a natural biomaterial for the design of nanoparticulate decoys that can effectively bind and clear the pathological antibodies responsible for ITP (Fig. 1). The binding capacity and specificity of platelet membrane-coated nanoparticles (PNPs) were evaluated before studying the neutralization capacity of PNPs against anti-platelet antibodies both *in vitro* and *in vivo*. Finally, an antibody-induced murine model of ITP was employed in order to assess treatment efficacy. As a possible new treatment for ITP, PNP administration holds distinct advantages compared to current therapies. By using the natural substrate of the pathological agent, the treatment is highly specific, which may prevent the immune compromising side effects commonly seen with other treatments. Further, by diverting anti-platelet antibodies away from healthy platelets, PNPs directly act to preserve normal hemostatic function. Ultimately, employing this biomimetic nanoparticle system for the specific treatment of ITP may serve to improve patient outcomes in the clinic.

2. Materials and methods

2.1. Animals

Male CD-1mice (6-week old; 20–24 g body weight) were purchased from Harlan Laboratories. All animal experiments were performed in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, San Diego.

2.2. Platelet isolation and membrane derivation

Whole blood was collected from male adult CD-1 mice (Harlan Laboratories) via puncture of the submandibular vein with ethylenediaminetetraacetic acid (EDTA; USB Corporation) as the anticoagulant. To isolate platelets, the blood was first centrifuged at 300*g* for 5 min at room temperature. The supernatant then was collected and spun at the same speed for another 5 min. The resulting supernatant, representing a platelet rich plasma, was then centrifuged at 2000*g* for 4 min in order to pellet down the platelets, which were resuspended in water, aliquoted, and stored at −80 °C for further use. Platelet membrane was derived by a repeated freeze-thaw process. A frozen aliquot of purified platelets was allowed to thaw at room temperature, centrifuged at 21,000*g* for 7 min, and the pellet was resuspended in water. The platelet suspension was refrozen, and the process was repeated three times. The pellet was finally resuspended in water, and the membrane protein concentration was quantified using a commercial BCA assay (Pierce).

2.3. Preparation and characterization of platelet membrane-coated nanoparticles (PNPs)

PNPs were prepared using a previously reported sonication method [23]. Polymeric nanoparticle cores were prepared using carboxyl acid-terminated 0.67 dL/g 50:50 poly($_{DL}$-lactic-*co*-glycolic acid) (PLGA; LACTEL Absorbable Polymers) in a nanoprecipitation process. A volume of 1 mL of a 10 mg/mL PLGA solution in acetone was added rapidly to 4 mL of water. The acetone was then allowed to evaporate under vacuum for 3 h. PNPs were prepared by fusing platelet membrane onto PLGA cores via sonication using a Fisher Scientific FS30D bath sonicator at a frequency of 42 kHz and a power of 100 W for 2 min. The size and zeta-potential of PNPs were measured by dynamic light scattering (DLS) using a Malvern ZEN 3600 Zetasizer. To study the morphology of PNPs by transmission electronic microscopy (TEM), samples were deposited onto a 400-mesh carbon-coated copper grid (Electron Microscopy Sciences) and negatively stained with vanadium (Abcam).

2.4. Platelet membrane to nanoparticle core ratio optimization

To optimize the platelet membrane to PLGA core ratio, PNPs were synthesized at membrane-to-core weight ratios ranging from 0.125 to 2 at a final polymer concentration of 1 mg/mL. PLGA cores without membrane coating were included as a control. The sizes of each set of particles were first measured by DLS immediately after synthesis. Afterwards, the particle solutions were adjusted to 1 × PBS by adding an equal volume of 2 × PBS and the particle sizes were measured again. An increase in size upon introduction of PBS was used as an indicator of particle instability.

2.5. In vitro binding capacity and specificity studies

To evaluate the *in vitro* binding capacity of PNPs, 10 μg of the nanoparticles was mixed with different amounts (2, 4, 8, 16, 32, 64, and 128 μg) of fluorescein isothiocyanate (FITC)-labeled polyclonal anti-mouse thrombocyte antibodies (Lifespan Biosciences). The precise antigen specificity of the antibodies was unknown. After mixing the PNPs with antibodies, the fluorescence intensity of the fluorescently labeled antibody was measured using a Tecan Infinite M200 plate reader. The mixtures were incubated for 10 min at 37 °C, then centrifuged at 21,000*g* for 8 min to pellet the PNP/anti-platelet complexes. The fluorescence intensity of the supernatant was measured and used to calculate the amount of antibody that had bound to the PNPs. To evaluate binding specificity, either 10 μg of PNPs or 10 μg of polyethylene glycol-functionalized nanoparticles (PEG-NPs) [25] were mixed with 32 μg of FITC-labeled antibody. To test the binding capacity in serum, 10 μg of PNPs