Journal of Controlled Release 210 (2015) 39–47

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Flow shear stress differentially regulates endothelial uptake of nanocarriers targeted to distinct epitopes of PECAM-1

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ARTICLE INFO

Article history:
Received 14 January 2015
Received in revised form 4 May 2015
Accepted 6 May 2015
Available online 9 May 2015

Keywords:
Intracellular delivery
Endothelial cells
Vascular immunotargeting
Cell adhesion molecules
Endocytosis
Fluid shear stress

ABSTRACT

Targeting nanocarriers (NC) to endothelial cell adhesion molecules including Platelet-Endothelial Cell Adhesion Molecule-1 (PECAM-1 or CD31) improves drug delivery and pharmacotherapy of inflammation, oxidative stress, thrombosis and ischemia in animal models. Recent studies unveiled that hydrodynamic conditions modulate endothelial endocytosis of NC targeted to PECAM-1, but the specificity and mechanism of effects of flow remain unknown. Here we studied the effect of flow on endocytosis by human endothelial cells of NC targeted by monoclonal antibodies Ab_{62} and Ab_{37} to distinct epitopes on the distal extracellular domain of PECAM. Flow in the range of 1–8 dyn/cm^2, typical for venous vasculature, stimulated the uptake of spherical Ab/NC (~180 nm diameter) carrying ~50 vs 200 Ab_{62} and Ab_{37} per NC, respectively. Effect of flow was inhibited by disruption of cholesterol-rich plasmalemma domains and deletion of PECAM-1 cytosolic tail. Flow stimulated endocytosis of Ab_{62}/NC and Ab_{37}/NC via eliciting distinct signaling pathways mediated by RhoA/ROCK and Src Family Kinases, respectively. Therefore, flow stimulates endothelial endocytosis of Ab/NC in a PECAM-1 epitope specific manner. Using ligands of binding to distinct epitopes on the same target molecule may enable fine-tuning of intracellular delivery based on the hemodynamic conditions in the vascular area of interest.

1. Introduction

Cellular uptake of targeted nanocarriers (NC) for drug delivery is regulated by parameters of carrier design (e.g., selection of target epitopes), as well as target cell phenotype and factors associated with the cellular microenvironment [1–4]. Thus, experimental models for delivery of NC should account for target cell conditions in vivo [5–8]. For example, the functional status of endothelial cells lining the vascular lumen, an important target for drug delivery, is greatly influenced by fluid shear stress of blood flow that varies under physiological and pathological conditions [9]. The role of blood rheology and hydrodynamics in NC binding to endothelium is extensively studied [10–15]. In contrast, relatively little is known about the role of these factors in the intracellular uptake of nanoparticles bound to specific endothelial surface molecules. Several lines of evidence suggest an important role of flow in the regulation of endocytosis of macromolecules and particles, such as albumin, non-targeted nanoparticles (e.g., quantum dots, SiO_2^- nanoparticles [16]), and nano- and micro-sized hydrogel spheres [17].

However, the role of hemodynamics in endocytosis of NC targeted to endothelial cells by affinity ligands including antibodies (i.e., Ab/NC) remains enigmatic. It is plausible that flow regulates this process in a ligand-specific fashion, since nature of the binding site and mode of ligand engagement control the mechanism of endocytosis. Recent studies in vitro and in vivo revealed that flow conditions modulate endothelial endocytosis of Ab/NC targeted to the cell adhesion molecules ICAM-1 and PECAM-1 [12,18]. Drug delivery using Ab/NC targeted to these determinants improves therapeutic effects of experimental drugs and biotherapeutics in animal models [19–22]. This justifies efforts directed towards extending our knowledge of the factors controlling intracellular delivery of NC targeted to these molecules. PECAM-1 antibodies bind to endothelial cells but do not accumulate significantly in the intracellular compartments [23,24]. In contrast, the multivalent binding of NCs coated with PECAM-1 antibody (e.g., Ab/NC) leads to intracellular uptake mediated by the pathway known as CAM-endocytosis, distinct from clathrin- or caveolae-mediated endocytosis, phagocytosis and macropinocytosis [23,25]. Furthermore, recent studies showed that shear stress stimulates endocytosis of PECAM-1-targeted Ab/NC [18].

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http://dx.doi.org/10.1016/j.jconrel.2015.05.006
0168-3659/

However, previous studies revealed that under standard static cell culture conditions, human endothelial cells differentially internalize Ab/NC targeted to specific PECAM-1 epitopes: e.g., they internalize Ab/NC targeted by monoclonal antibody 62 (Ab_{62}) but not by monoclonal antibody 37 (Ab_{37}), which both are directed to distinct epitopes located in the distal Ig-like domain of PECAM-1 (i.e., Ab_{62}/NC and Ab_{37}/NC, respectively) [26]. These findings imply that control of endothelial internalization by physiological factors including flow may be different for Ab/NC targeted to distinct epitopes of PECAM-1. In the present study we have investigated whether this effect of flow is epitope-specific.

2. Results

2.1. Flow differently modulates endothelial internalization of Ab/NC targeted to distinct PECAM-1 epitopes

Binding to and uptake by target cells are proportional to Ab/NC's avidity, controlled by antibody affinity and number on a NC surface. Coupling ~200 antibody molecules per 100 nm particle provides nearly maximal surface density of monolayer IgG coating [27]. We started with assessing the uptake by endothelial cells of such NC coated by Ab_{37} vs Ab_{62}, either at static conditions or 30 min after exposure to non-pulsatile laminar flow generating a flow shear stress of 4 dyn/cm^2. Double-label fluorescent microscopy with secondary fluorescent antibody allows to distinguish cell surface-bound vs intracellular fluorescent nanocarriers (Fig. 1A). We found that flow almost doubles uptake of Ab_{37}/NC, which are barely internalized by static endothelial cells (Fig. 1A & B). However, flow rather trivially augmented uptake of Ab_{62}/NC, which are effectively internalized by static cells (Fig. 1A & B).

The binding of Ab_{37}/NC to endothelial cells was markedly lower than that of Ab_{62}/NC (Supplemental table 2 and Fig. 1A and C). Noteworthy, flow stimulated internalization of Ab_{37}/NC without changing its binding (Fig. 1B and C). The data of uptake of Ab/NC incubated at different concentrations with static cells further distinguished binding vs internalization. As expected, endothelial binding of both types of Ab/NC increased proportionally to their concentration (Fig. 2A). Importantly,

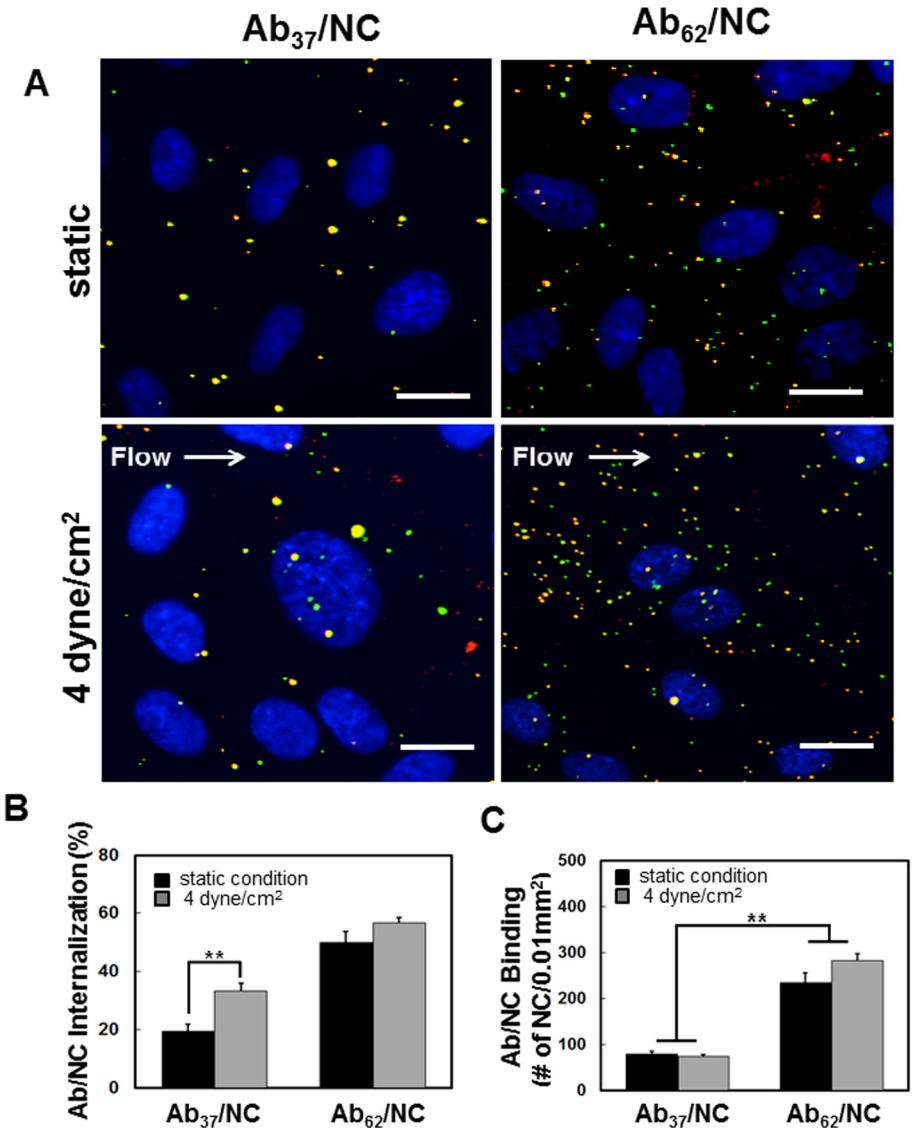

Fig. 1. Endothelial binding and internalization of nanocarriers coated by Ab_{37} and Ab_{62}. *A*, Representative fluorescence images showing endothelial binding (total particles) and internalization (green particles) of NC coated with Ab_{37} (left panel) versus Ab_{62} (right panel) at maximal Ab density on the surface of NC (200Abs/NC) under static and laminar flow conditions. Scale bar is 20 μm. *B* and *C*, confluent endothelial cells were incubated or exposed to flow (4 dyn/cm^2) with Ab/NC at the NC concentration of 2×10^9/ml for 30 min at 37 °C. Flow stimulates internalization of Ab_{37}/NC (*B*). Fewer Ab_{37}/NC were bound to endothelial cells under static and flow conditions (*C*). The percentage of Ab/NC internalized into endothelial cells (*B*) and total number of Ab/NC bound to endothelial cells (*C*) in each image field (0.01 mm^2) were quantified by fluorescence microscopy and presented as Mean ± S.E. (n = 8). $^*p < 0.05$, $^{**}p < 0.01$.