Mechanism of Nanocarrier Release from the Abluminal Endothelium

R. L. Manthe1, S. Muro1,2

1Fischell Department of Bioengineering and 2Institute for Bioscience and Biotechnology Research, University of Maryland, College Park, MD, 20742, USA
rmanthe@umd.edu

ABSTRACT SUMMARY

Passage through the endothelial barrier that lines the vasculature represents a major obstacle for effective drug delivery to certain tissues, e.g., the central nervous system. Targeting of drug carriers to endothelial receptors involved in vesicular transcytosis from the luminal (apical) to the abuminal (basolateral) side of said barriers is growing as a viable strategy to overcome this obstacle. However, the process by which a ligand-targeted carrier dissociates from its engaged receptor after transcytosis, to be released from the abuminal endothelial surface and penetrate a tissue, is rather unknown. In this study, we have examined this aspect using model polymer nanocarriers targeted to endothelial intercellular adhesion molecule-1 (ICAM-1) in a transwell model. Our results indicate that endothelial secretion of matrix metalloproteinases leads to receptor (ICAM-1) cleavage, thereby permitting release of the transcytosed carriers from the abuminal side of the endothelium.

INTRODUCTION

The endothelium represents a major biological barrier to the transport of therapeutic agents from the bloodstream into tissues requiring intervention (1). To overcome this obstacle, the field of drug delivery has focused on the design of nano-scale drug carriers that can target cell-surface receptors involved in vesicular-mediated transcytosis across this barrier (2). For example, the transferrin receptor, insulin receptor, LDL receptor family, and aminopeptidase P are common targets used for ligand-mediated drug delivery across the endothelial lining. These molecules rely on classical pathways, i.e., clathrin- or caveolae-mediated transcytosis, which have been shown to mobilize ligands below ~150 nm and ~70 nm, respectively.

Alternatively, clathrin- and caveolae-independent pathways seem to offer an opportunity to traverse endothelial linings. This is the case for carrier targeting to intercellular adhesion molecule-1 (ICAM-1). ICAM-1 is a transmembrane glycoprotein overexpressed on the endothelium during many pathological states, where it acts as a counter-receptor to integrins on leukocytes, contributing to their transmigration across the endothelium and into tissues (3). ICAM-1-targeting has recently been shown to provide transcytosis of ICAM-1-targeted polymer nanocarriers across model endothelial barriers (4). This is regulated via CAM-mediated endocytosis, a clathrin- and caveolae-independent pathway which provides transport of drug carriers within a wide range of sizes (e.g., 100 nm to 5 µm) (5).

However, despite this knowledge and technological advances, the means by which ligand-targeted carriers (which bind with significant avidity to their receptors) are released from their receptors after being transported to the abuminal endothelial surface vs. remaining bound to their receptors at this interface, is not understood. A possibility is that a ligand-targeted carrier disengages its receptor while in transit across the endothelial cell. This would be possible if the ligand-receptor complex travels through acidic or degradative compartments which may disrupt the ligand-receptor bond. However, transcytosis implies avoidance of intracellular lysosomal compartments, which are typically associated with such features. Therefore, it is likely other mechanisms may contribute to this, representing an important question for the field of drug delivery and basic biology on transcytosis.

Proteolytic cleavage of ligand-receptor complexes following transcytosis (hence, extracellularly) could be a potential mechanism for carrier release from the abuminal endothelial surface. In particular, matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptides secreted by endothelial cells and other cell types, are known to process a number of bioactive molecules, including the cleavage of cell-surface receptors, in order to regulate barrier function for leukocyte recruitment and transmigration across endothelial surfaces (6). The gelatinase subfamily of MMPs, consisting of MMP-9 and MMP-2, seem especially critical to this process. Furthermore, in the case of ICAM-1, literature indicates that there is significant interplay between this receptor and MMPs in the inflammatory response. Specifically, ICAM-1 engagement has been shown to stimulate MMP expression (7), and MMP-9 has been documented to cleave the extracellular domain of ICAM-1, leading to release of this receptor from the cell surface (8).

As an example to illustrate the potential mechanism underlying effective release of transcytosed carriers from the abuminal endothelial surface, we have examined in this work the possibility of MMP-9 and MMP-2 being involved in ICAM-1 cleavage and effective transcytosis of ICAM-1-targeted nanocarriers.

EXPERIMENTAL METHODS

Model polymer nanocarriers were prepared by surface adsorption of anti-ICAM onto 100 nm-diameter polystyrene latex beads (anti-ICAM NCs), yielding ~250 nm carriers. Non-degradable polystyrene models were used to avoid potential confounding effects of concomitant carrier degradation. This model has been extensively validated in the past (9).
Human umbilical vein or human brain endothelial cells (HUVECs or HBMECs, respectively) were grown to confluence on 1.0 μm-pore transwell inserts separating an apical chamber facing the luminal endothelial surface vs. a basolateral chamber facing the abluminal endothelial surface. Cells were incubated overnight with TNFα to mimic a pathological state, where targeted carriers may be employed for drug therapy. Anti-ICAM NCs were then added to the upper chamber over the cells and incubated for 30 min at 37°C to permit carrier binding to the luminal cell surface, followed by removal of non-bound carriers and incubation in the absence of carriers for an additional 4.5 h, to allow for uptake of bound carriers and transcytosis.

To measure the release of MMP-9, MMP-2, and the cleaved extracellular domain of ICAM-1, cell culture supernatants from the transwell apical and basolateral chambers were collected and analyzed by ELISA.

For assessment of anti-ICAM NC transport through endothelial monolayers, anti-ICAM was labeled with a radioisotope (125Iodine) and measured in the basolateral chamber (transcytosed fraction) vs. the transwell filter (cell fraction) using a gamma counter.

The contribution of MMPs to ICAM-1 cleavage and carrier transcytosis was further tested using MMP inhibitors or addition of recombinant MMPs.

RESULTS AND DISCUSSION

First, endothelial cells grown on transwell membranes secreted both MMP-9 and MMP-2 under inflammatory-like conditions. This correlated well with release of the extracellular domain of ICAM-1 from these cells, as reported, validating our model. Importantly, MMP-9 was mostly released to the apical chamber above the cells (92% of total release), while MMP-2 appeared similarly in both chambers.

Next, we evaluated if release of these endothelial markers was also found in pathologically altered cells incubated with anti-ICAM NCs. This was the case: both MMP-9 and MMP-2 where released from cells, yet, their distribution was different from that of cells incubated in the absence of anti-ICAM NCs. Importantly, the presence of carriers rapidly shifted release of MMP-9 from the apical to the basolateral chamber (from 8% to 83% of total release by 30 min), and a similar, yet more slow, outcome was observed for MMP-2 (from 44% to 85% of total release by 5 h). In addition, cleaved ICAM-1 was released from the abluminal cell surface at a much greater level than the luminal surface (4-fold) by 5 h. In this model, 32% of anti-ICAM NCs were transported across the endothelial monolayer by 5 h. This agrees with our hypothesis that release of anti-ICAM NCs from the abluminal endothelial cell after transcytosis may be regulated by MMP-mediated ICAM-1 cleavage.

Validating this, MMP-9 inhibitor caused a 1.5-fold reduction in the release of cleaved ICAM-1 from the abluminal endothelial surface. Also, inhibition of MMP-9 rendered greater retention of anti-ICAM NCs in the cell-associated fraction compared to the control (20% increase) and a 1.6-fold decrease in the number of anti-ICAM NCs transported from the luminal to abluminal cell surface. Similarly, we observed a slight reduction in the release of cleaved ICAM-1 from the abluminal surface in the presence of an MMP-2 inhibitor compared to the control (20% decrease), along with greater carrier retention in the cell fraction (27% increase), and a 2.4-fold decrease in carrier release in the basolateral chamber compared to the control. Furthermore, reduction in carrier transport was even more pronounced by inhibiting both MMP-9 and MMP-2, with a ~31% increase in retention of carriers in the cell fraction and a concomitant 3-fold reduction in carrier release in the basolateral chamber.

Finally, we investigated whether basolateral release of anti-ICAM NCs transported across endothelial cells could be enhanced by the addition of recombinant MMPs. Using MMP-9 as an example, addition of exogenous enzyme to endothelial cells resulted in a 1.6-fold increase in carrier detection in the basolateral chamber, concurrent with a 1.4-fold decrease in the number of carriers retained in the cell fraction.

CONCLUSION

Overall, our results indicate that endothelial secretion of MMP-9 and MMP-2 contributes to cleavage and release of the extracellular domain of ICAM-1, thereby permitting the release of ICAM-1-targeted NCs from the abluminal surface of endothelial cells after transcytosis across said monolayers. This is the first time that the mechanism of abluminal release of carriers transcytosed via ligand-receptor mediated interactions is elucidated. As such, this study provides critical insight into fundamental pathways underlining the biology of transcytosis and their potential translational applications, e.g. optimization of ligand (ICAM-1)-targeted drug carriers for enhanced delivery across endothelial barriers, critical for a significant number of therapeutic interventions such as those requiring transport across the blood-brain interface.

REFERENCES


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