Published in final edited form as:
Semin Thromb Hemost. 2010 April ; 36(3): 332–342. doi:10.1055/s-0030-1253455.

Targeting Antioxidant and Antithrombotic Biotherapeutics to Endothelium

Ronald Carnemolla, Ph.D.[1], **Vladimir V. Shuvaev, Ph.D.**[1], and **Vladimir R. Muzykantov, M.D., Ph.D.**[1]

[1] Department of Pharmacology and Program in Targeted Therapeutics of the Institute for Translational Medicine and Therapeutics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Abstract

The endothelium is one of the key targets for pharmacological interventions in oxidative stress and thrombosis, two conditions that are notoriously difficult to treat due to limited efficacy and precision of action of current drugs. Design of molecular and nano-devices that deliver potent antioxidant and antithrombotic therapeutic enzymes to the endothelium holds promise to improve the potency, localization, timing, specificity, safety, and mechanistic precision of these interventions. In particular, cell adhesion molecules expressed on the surface of resting and pathologically altered endothelial cells can be used for drug delivery to the endothelial surface (preferable for thrombolytics) and into intracellular compartments (preferable for antioxidants). Drug delivery platforms including protein conjugates, recombinant fusion constructs, and stealth polymer carriers designed to target these drugs to endothelium are reviewed in this article.

Keywords

Targeted therapeutics; nanocarriers; drug delivery; antioxidants; thromboprophylaxis; endothelium

The endothelium represents an important therapeutic target in thrombosis and vascular oxidative stress. Advances in molecular biology and biotechnology paved the way for the design of potent and specific recombinant therapeutic proteins including antithrombotic and antioxidant enzymes. Thus plasminogen activators (PAs) catalyze dissolution of fibrin clots providing re-perfusion, whereas superoxide dismutase (SOD) and catalase detoxify reactive oxygen species (ROS) that may inflict tissue damage and inflammation. These enzymes represent labile, yet potentially advantageous biotherapeutic agents that require precise delivery to desired sites of action in the target cells (i.e., endothelial luminal surface for fibrinolytics and intracellular compartments for antioxidants).

Unfortunately, these biotherapeutics have no endothelial affinity and their delivery to the desired site of their action is inadequate; hence adverse effects limit clinical utility of fibrinolytic PAs and inadequate effectiveness precludes the use of antioxidant enzymes. In theory, medical application of these biotherapeutics may benefit from targeted delivery to selected compartments in endothelium. Endowing these agents or their carriers with an affinity to endothelial cells that provides targeted delivery to either the endothelial lumen or its intracellular compartments holds promise to localize therapeutic interventions in desired

Address for correspondence and reprint requests: Vladimir R. Muzykantov, M.D., IFEM, University of Pennsylvania Medical Center, 1 John Morgan Building, 3620 Hamilton Walk, Philadelphia, PA 19104-6068 (muzykant@mail.med.upenn.edu).

endothelial compartments in vascular areas of interest and optimize treatment of these vascular disorders. This article briefly discusses the current status of the experimental design and development of targeted delivery of antioxidants and fibrinolytics to the endothelium.

MOLECULAR TARGETS FOR ENDOTHELIAL DRUG DELIVERY

Ideally, binding of targeted drugs to the target should cause therapeutically beneficial side effects and provide the proper subcellular localization of a drug. Depending on the therapeutic goal, a drug should either be retained on the cell surface or traffic to a proper subcellular compartment. Binding of targeted drugs may activate endothelial cells or induce shedding and/or internalization of target determinants, change their functionality, or otherwise disturb the endothelium. Obviously, targeting of antioxidant or antithrombotic agents should not cause harmful side effects. In this aspect, targeting protective drugs is different versus targeting toxic agents to tumor endothelium. In the latter scenario, toxic effect to the target cells is a bonus. Therefore, the specificity of tumor targeting must be maximal to avoid collateral damage. In contrast, endothelial disturbance by targeted antioxidant and antithrombotic drugs must be minimized to avoid aggravation of oxidative stress, inflammation, and thrombosis. However, the criteria of specificity of targeting are less stringent in this case because drugs alleviating these conditions (often associated with systemic pathologies) are less likely to cause systemic harmful effects; thus pan-endothelial delivery of antioxidants or antithrombotic therapeutics throughout the vasculature is a suitable option. Of note, the pulmonary vasculature is the major capillary network, containing ~30% of the endothelial surface in the body and receiving the entire cardiac output. As a result, agents with an endothelial affinity accumulate in the lungs after intravenous (IV) injection, even if their target determinants are relatively evenly distributed throughout all types of endothelial cells in the body.[1] This vascular bed is an important target for treatment of acute lung injury, oxidative stress, thrombosis, and inflammation, among other conditions.

Numerous molecules localized on the surface of endothelial cells of diverse phenotypes have been identified by high-throughput approaches[2] including selective proteomics of the endothelial plasmalemma[3,4] and in vivo phage display[5] and low throughput approaches including immunological techniques.[1] Specific domains in the endothelial plasmalemma are enriched in certain molecules. For example, rat glycoprotein GP85 is predominantly localized on the luminal surface of the plasmalemma domain and belongs to a thin part of the endothelial cell body that lacks major organelles and separates alveolar and vascular compartments.[6] Antibodies to GP85 accumulate in rat pulmonary vasculature without internalization and deliver conjugated tissue plasminogen activator (tPA) into the pulmonary vasculature.[7] A human counterpart of this antigen could be an interesting candidate for drug delivery to the surface of alveolar capillaries. Further, determinants in the caveoli provide effective intracellular and transendothelial delivery of antibodies and small protein conjugates.[8] However, utility of the caveolar determinants for sustained anchoring of drugs on the cell surface as well as for targeting carriers larger than 50 to 80 nm is questionable. Surface density of some endothelial molecules is reduced upon disease conditions associated with oxidative stress and ischemia, hindering targeting to these determinants.[9]

This section provides a brief overview of endothelial targets suitable for delivery of antioxidant and antithrombotic agents (Table 1). For example, angiotensin-converting enzyme (ACE) is a transmembrane constitutive glycoprotein preferentially expressed at the endothelial luminal surface in pulmonary capillaries.[9–11] ACE converts Ang I into Ang II, a vasoactive peptide that exerts vasoconstricting, prooxidant, prothrombotic, and proinflammatory activities.[12] Endothelial cells internalize ACE antibodies (anti-ACE) and anti-ACE conjugates.[13] Labeled anti-ACE selectively accumulates in the lungs after IV

injection in rats, mice, cats, primates, and humans.[11,14–16] Pilot tests did not reveal harmful effects of anti-ACE in animals[10,17] and humans.[17] Anti-ACE formulations are being used for targeting drugs including antioxidants and fibrinolytics to the pulmonary endothelium in animals.[18–20] Ischemia, oxidants, cytokines, and other pathological agents suppress ACE expression and thus inhibit targeting to ACE.[21,22]

Platelet-endothelial adhesion molecule (PE-CAM)-1, CD31 and intercellular adhesion molecule (ICAM)-1, CD54 are transmembrane glycoproteins constitutively expressed on endothelial cells throughout the vasculature. Their ligands (e.g., anti-ICAM and anti-PECAM) infused locally in a conduit artery accumulate in the downstream vascular areas including cardiac,[23] cerebral,[24] and mesentery[25,26] vasculature. Anti-PECAM and anti-ICAM tend to accumulate in the lungs after IV injection and may be used for drug targeting to either normal and/or pathologically altered pulmonary and systemic endothelium.[27] PECAM and ICAM are involved in mechanisms of cellular recognition, adhesion, and transendothelial migration of leukocytes.[28] Thus drug targeting to these molecules may inhibit leukocyte trafficking.[29] Leukocyte infiltration is generally viewed as an injurious factor,[30] and attenuation of leukocyte infiltration would be a bonus in the treatment of inflammation. Endothelial cells do not internalize anti-ICAM and anti-PECAM or their monovalent fragments, but they do internalize multimeric, multivalent conjugates causing excessive cross-linking of ICAM and PECAM.[31,32] Therefore, based on the molecular design of the conjugates or fusion proteins targeted to these cell adhesion molecules, either intracellular or surface addressing of the cargoes is possible.

PECAM is stably expressed on the endothelium at the level of a million copies per cell predominantly localized in interendothelial borders.[33] Multivalent binding of PECAM-targeted nanocarriers offers effective means for endothelial drug delivery.[11,32] Diverse reporter,[34] enzymatic,[23,35,36] and genetic materials[37] conjugated to anti-PECAM accumulate and display their functional activity in the endothelium as soon as 10 minutes after IV injection in mice, rats, and pigs.

In contrast to constitutively and stably expressed PECAM-1, quiescent confluent endothelial cells in culture do not express appreciable amounts of ICAM-1, but expression is markedly increased after cytokine treatment.[38] Endothelial cells in the vasculature express ICAM-1 at a surface density of 2×10^4 to 2×10^5 surface copies per cell, and this level doubles upon proinflammatory challenge.[39] Other cell types also express ICAM, yet the blood-accessible ICAM is located predominantly on endothelial cells. Anti-ICAM and anti-ICAM conjugates bind to the vascular endothelium after IV administration in animals.[40,41] Pathological stimuli including oxidants, cytokines, and abnormal shear stress stimulate de novo synthesis and surface expression of ICAM by endothelial cells[42] and thereby facilitate anti-ICAM endothelial targeting.[40,41,43,44] Conjugation of anti-ICAM to therapeutics,[41,45] liposomes,[46] or polymer carriers[26] providing multivalent binding to the endothelium further enhances drug delivery.

Vascular cellular adhesion molecule (VCAM)-1) and selectins are normally absent on the vascular lumen but are exposed on pathological endothelium. For example, pathological mediators cause mobilization of intracellular P-selectin to the endothelial surface within 10 to 30 minutes[30] and induce de novo synthesis and surface expression of E-selectin[47] and VCAM-1[33] within several hours. Selectins and VCAM-1 facilitate adhesion of leukocytes to endothelial cells.[48] Selectin and VCAM-1 ligands represent attractive affinity moieties for delivery diagnostics and therapeutic agents to activated endothelium. Experiments in cell culture and animals show that anti-selectins permit drug targeting to cytokine-activated endothelium.[49,50] Endothelial cells constitutively internalize selectins via clathrin-coated pits,[51–53] permitting entry into endothelial cells of anti-E-selectin targeted liposomes,[54] anti-

inflammatory drugs,[54,55] and genetic materials.[56] Yet, even at maximal activation, selectins and VCAM-1 are exposed at surface densities lower than PECAM-1 and ICAM-1; hence robustness may be suboptimal for therapies requiring delivery of large doses of drugs. P-selectin targeted compounds also bind to activated platelets.[57] E-selectin and VCAM-1 seem to be more readily expressed in activated endothelium outside the pulmonary vasculature (e.g., in arteries and skin microvasculature).[58] These determinants seem to be useful for diagnostic visualization of activated endothelium in inflammation foci by delivery of conjugated isotopes[59] or ultrasound contrasts.[57,60]

TARGETING OF ANTIOXIDANT ENZYMES

Cells normally produce low levels of ROS such as superoxide anion and hydrogen peroxide, but excessive vascular ROS generation is postulated to underlie the pathogenesis of many diseases.[61] Both acute (acute lung injury, ischemia/reperfusion injury, inflammation, and complications of organ transplantation) and chronic (e.g., hypertension, diabetes, and atherosclerosis) vascular disorders are associated with abnormally high level of ROS produced by activated leukocytes and endothelial cells.[62] Therefore, the endothelium is an important target for antioxidant interventions.

Superoxide anion O_2^- is not particularly toxic by itself, but at excessive levels can yield a highly reactive and damaging hydroxyl radical OH. Further, it consumes a protective agent nitric oxide (NO·) in a fast reaction producing a strong oxidant peroxynitrite that aggravates vascular oxidative stress, inflammation, vasoconstriction, and thrombosis.[61,63] Superoxide dismutases (SODs) are metal-containing enzymes that catalyze superoxide conversion into H_2O_2, thus alleviating these pathological mechanisms.[64] There are three types of human SOD: cytosolic CuZnSOD (SOD1), mitochondrial MnSOD (SOD2), and extracellular SOD (SOD3).[64,65] Catalase, a tetrameric enzyme localized in the cytosol and intracellular vesicles called peroxisomes, decomposes H_2O_2 into water and oxygen.

Use of SOD and catalase for antioxidant interventions was proposed almost four decades ago.[66] However, their medical utility is precluded by rapid elimination and inadequate delivery to target cells.[67] Diverse modifications of SOD and catalase including coupling with polyethylene glycol or other chemicals and encapsulation into liposomes have been designed to solve this problem (Table 2).[68–70] Some of these derivatives and SOD mimetics showed protective effects after intravascular or local administration in animal models of systemic or focal oxidative stress, respectively.[68,71–73] There were also attempts to optimize cellular binding of SOD and catalase. For example, lecithinized SOD (PC-SOD) binds to some cell types including endothelial cells in vitro and was protective in several animal models of human pathologies including myocardial infarction, colitis, and tumor growth.[74–76] SOD conjugation with folate enhances enzyme uptake by macrophages.[77] A recombinant fusion of mitochondrial MnSOD (SOD2) and heparin-binding domain of EC-SOD (SOD3) has been synthesized[78]; this SOD2/3 chimera binds to cellular glycocalyx and alleviates vascular dysfunction in models of myocardial ischemia.[79,80] Other SOD fusion proteins have also been reported (e.g., SOD-hemoglobin fusion[81] and bacterial Fe-SOD fused with a single-chain variable fragment (scFv) LC-1 antibody to lung cancer cells[82]).

However, the pharmacokinetics and specificity of endothelial binding of these antioxidant formulations remain suboptimal and did not provide controlled delivery into these cells, the goal that seems necessary for interception of intracellular ROS.[83] Inadequate delivery precludes the therapeutic use of catalase and SOD. Alas, despite remarkable efforts to improve their pharmacokinetics,[84] effective and specific endothelial delivery remain elusive.

Targeting catalase and SOD to specific endothelial targets may help to solve this problem.[31,32] Conjugates with antibodies to ACE,[18] PECAM,[85] and ICAM[19] specifically

bind to endothelium in vitro and in vivo and accumulate in the pulmonary and systemic endothelium after IV injection. Multivalent catalase conjugates with anti-ICAM or anti-PECAM,[18,19,86,87] as well as catalase loaded to polymer nanocarriers coated by these antibodies enter endothelial cells.[38] Catalase delivered to endocytic vesicles degrades H_2O_2 and protects endothelial cells from oxidative stress.[38] IV injected anti-PECAM/catalase conjugates protect against acute oxidative stress in the pulmonary vasculature in mice,[86] reduce lung edema and improve blood oxygenation in a mouse model of pulmonary ischemia/reperfusion,[88] and improve the outcome of lung transplantation in rats.[89] IV injection of anti-ACE/catalase conjugate in a donor animal also protects lungs in a model of transplantation in rats.[20] Similarly, multivalent anti-PECAM/SOD conjugates bind to endothelial cells with high affinity and specificity and protect cells against oxidative stress caused by superoxide anion.[90] Further, IV-injected anti-PECAM/SOD binds to the endothelium and alleviates superoxide-mediated oxidative stress in blood vessels and vascular contractility in animals.[88]

Of note, targeting SOD and catalase to the same endothelial determinant, PECAM-1, provides different and specific protective effects in several animal models of acute vascular oxidative stress, emphasizing importance of interception of specific ROS in given pathologies.[88] Of note, lysosomal degradation of antioxidant enzymes[91] terminates the protective effect within a few hours after internalization.[92] Drugs affecting lysosomal trafficking and degradation prolongs the protective effects of anti-ICAM/catalase formulations.[91,92] Further, using catalase encapsulated into PECAM-targeted bio-compatible polymer nanocarriers selectively permeable to H_2O_2, but not to proteases, permits a prolonged antioxidant effect. A freeze-thaw modified double emulsion allows encapsulation of active catalase into such protective nanocarriers with controlled size and shape, appropriate for vascular delivery into endothelial cells.[93,94] Typical carrier morphologies, controlled by chemistry of the polymers utilized, are spheres with diameters <500 nm[93] or flexible filaments that are a few microns in length and ~50 nm in cross section.[94] Catalase-loaded polymer spherical nanocarriers targeted to PECAM-1 deliver the enzyme to the pulmonary vasculature after IV injection in animals and protect endothelial cells against H_2O_2-induced injury for an extended period.[95]

ENDOTHELIAL TARGETING OF ANTITHROMBOTIC DRUGS

Thrombosis is caused by intravascular blood clots. Pathologically altered vasculature (e.g., due to oxidative stress, inflammation, or ischemia) is predisposed for thrombosis, in part due to suppression of natural antithrombotic mechanisms in endothelium.[96] It is tempting to postulate that anchoring of exogenous antithrombotic proteins on the endothelial lumen may help to compensate for this dysfunction and thereby improve management of thrombosis.

Fibrinolytic PAs convert plasminogen to plasmin that in turn cleaves fibrin facilitating clot degradation and restoration of blood perfusion. These drugs are used in clinics for urgent fibrinolytic therapy but are marred by hemorrhagic and other side effects, due to inadequate delivery to the therapeutic site. Attempts to improve delivery by conjugating PAs with antibodies to clot components have not yielded decisive improvement in therapy, likely due to impermeability of clots.[97,98] None of the current PA formulations are useful for short-term thromboprophylaxis despite the urgent and critical un-met medical need for such intervention in identified patient cohorts.

Theoretically, enhancing antithrombotic activity on the vascular luminal surface could be used for thromboprophylaxis. Gene transduction of antithrombotic proteins in the vasculature of laboratory animals supports this notion,[99] but this approach is impractical in acute settings. It is conceivable that immunotargeting of antithrombotic proteins to the

endothelial surface would rapidly restrict and sustain their activity to the vascular lumen. This approach can potentially provide thromboprophylaxis in patients with a high propensity for thrombosis, particularly in settings where the risk of bleeding prohibits use of systemic anticoagulation.[100]

Several endothelial determinants have been employed for this function. For example, tPA chemically conjugated with anti-ACE retained fibrinolytic and antigen-binding activities and exhibited sustained preferential accumulation in rat pulmonary vasculature.[101] Other endothelial determinants have also been explored for this goal; both tPA and urokinase-type PA (uPA) chemically conjugated with antibodies against antigens enriched in the pulmonary endothelium accumulated in the pulmonary vasculature.[102,103]

ICAM-1 and PECAM-1, stably expressed by endothelium and not supporting endocytosis of single antibodies, represent an attractive target for anchoring antithrombotic drugs on the endothelial luminal surface (Table 2). After IV injection in rats, pulmonary uptake of anti-ICAM/tPA conjugates is almost two orders of magnitude higher than that of control IgG/tPA, which resulted in enhanced fibrinolysis of subsequent pulmonary emboli.[104] Anti-ICAM carriers provide robust targeting to the pulmonary endothelium, on par with the best candidate carriers tested to date including antibodies to ACE and caveoli-associated antigens.[101,103] However, in contrast to these endothelial determinants, ICAM-1 offers enhanced pulmonary targeting in the context of inflammation that is frequently intertwined with thrombosis.[105,106] In addition, thrombin upregulates the expression of ICAM-1, which provides an additional rationale for its use as a target for delivering antithrombotic agents.[107]

Chemical conjugation of proteins with antibodies yields multivalent conjugates that cause endocytosis.[85] This is undesirable in the context of sustained retention of fibrinolytics on the endothelial lumen. In contrast, recombinant fusion of enzymes with genetically engineered antibody fragments, single-chain Fv (scFv, comprising variable domains of heavy chain V_H and light chain V_L) yields a monovalent (thus no internalization via ICAM or PECAM), homogeneous, and relatively small (50 to 70 kDa, hence low immunogenicity and lack of Fc-fragment mediated side effects) bifunctional recombinant drug. As a proof of principle for prophylactic thrombolysis by endothelium-targeted thrombolytic fusions, using a linker of three Gly_4Ser repeats, an anti-PECAM scFv was fused with uPA, which exists as an inactive single-chain zymogen of 411 amino acid residues (single-chain uPA, scuPA[108]). In the presence of fibrin, endogenous plasminogen activators convert plasminogen to plasmin, which in turn cleaves the Lys158-Ile159 peptide bond in scuPA and generates fully active two-chain uPA (tcuPA) consisting of two polypeptide chains linked by a Cys148-Cys279 disulfide bond.[109]

The natural scuPA consists of three domains: the N-terminal domain homologous to the epidermal growth factor, the kringle domain, and the C-terminal catalytic domain. Urokinase binding to a cellular receptor via the “growth factor-like domain” (GFD) activates vascular cells,[110] but deletion of the GFD provides a low molecular weight form of scuPA (MW 32 kDa) that has enzymatic features similar to those of full-length scuPA. After IV injection, the protein composed of an anti-PECAM scFv fused with lmw-scuPA (scFv/uPA) preferentially accumulated in the lungs of wild-type but not PECAM-deficient mice, persisted in the lungs for at least 3 hours and remained on the endothelial surface. Compared with nontargeted uPA, scFv/uPA augmented local lysis of pulmonary emboli in a mouse pulmonary thrombotic model.[108] Further, scFv/uPA accumulated in the cerebral vasculature after intra-arterial and IV injection, dissolved cerebral clots, and improved blood reperfusion without hemorrhagic complications, thereby mitigating postthrombotic brain edema in a mouse model of cerebral embolism.[24]

The scuPA zymogen (prourokinase) can be activated by trace amounts of plasmin over time, and formed tcuPA may cause adverse effects that would limit prophylactic use.[111] Because tcuPA is rapidly inactivated by plasminogen activator inhibitor (PAI-1), the duration and effectiveness of prophylaxis would also be limited. Further, thrombin inactivates uPA by cleaving Arg156-Phe157, negating its effect at sites of active thrombosis.[112] These problems might be solved by deleting Phe157 and Lys158, which yields a plasmin-resistant mutant activated by thrombin (uPA-T).[113] This pro-enzyme will not be activated by plasmin in vivo (thus avoiding systemic effects and premature PAI-1 inactivation), and thrombin will activate it locally at sites of nascent thrombosis within seconds of clotting. Replacing the native plasmin activation site in the uPA moiety of scFv/uPA with a thrombin activation site provided thrombin-activated anti-PECAM scFv/uPA-T.[114] This construct was also found to contain an intrinsic thrombin-sensitive cleavage site in the anti-PECAM scFv moiety, providing a built-in mechanism for local drug release. The scFv/uPA-T is latent and resists the PA inhibitor PAI-1 until activated by thrombin. After IV injection in mice, scFv/uPA-T did not consume plasma fibrinogen, in contrast with scFv/uPA that has this liability. However, scFv/uPA-T bound to the endothelium and accumulated in the vascularized organs, particularly the lungs. In a mouse model of thrombin-induced pulmonary thrombosis, scFv/uPA-T provided more potent and durable thromboprophylaxis than both plasmin-sensitive scFv/uPA and lmw-scuPA. Further, injection of mice with scFv/uPA-T prior to unilateral lung ischemia/reperfusion attenuated pulmonary fibrin deposition, to a significantly greater extent than plasmin-sensitive scFv/uPA, and restored arterial oxygen tension; PAI-1 susceptible plasmin-activated scFv/uPA did not improve blood oxygenation.[102]

The suppression of endothelial thrombomodulin activity (TM, a transmembrane glycoprotein that switches procoagulant and proinflammatory effects of thrombin to opposing effects via activation of protein C) is characteristic of acute lung injury, and some success has been achieved with a replacement therapy using soluble recombinant TM and activated protein C (APC).[115] Further, TM fused with a tissue factor antibody has potent antithrombotic activity in a rat model.[116] Yet these biotherapeutics seem unsuitable for prophylaxis due to fast disappearance from the vascular lumen. To solve this problem, a new anti-PECAM scFv/TM fusion has been produced and shown to bind and reside on endothelial surface, accumulate in the pulmonary vasculature, and attenuate thrombosis and tissue damage in mouse models of lung ischemia-reperfusion and endotoxin-induced acute inflammatory lung injury to a greater extent than nontargeted soluble TM, without causing bleeding (a known liability of APC treatment).[115]

In summary, endothelium-targeted thromboprophylaxis triggered by a prothrombotic enzyme illustrates a novel approach to time- and site-specific regulation of “on demand” reactions that can be modulated for therapeutic benefit. In clinical settings, this strategy of targeting antithrombotic drugs to the endothelial surface may provide local thromboprophylaxis in patients with an acute risk of developing new or recurrent thrombi and prevent clot extension.

CONCLUSION

Endothelial targeting of antioxidant and antithrombotic enzymes has been achieved in intact animals and animal models of human pathologies. Recent animal studies showed that ACE and especially ICAM and PECAM are good potential anchors for targeting these biotherapeutics to endothelium. Their functions are fairly well understood, which helps avoid unintentional side effects in a given pathological context. Careful selection of targets and modulation of features of the antibody-drug conjugates, such as valency and size, prodrug activation features, and encapsulation into protective polymeric carriers, provide

powerful tools for the control of subcellular addressing, activation, and duration of the effects of the enzymes.

Scaling up synthesis and quality control of targeted drug delivery systems with a standard, Food and Drug Administration acceptable level of homogeneity is a challenge for their industrial development and clinical utility. Recombinant mutant thrombolytic prodrugs fused with affinity peptides such as anti-PECAM or anti-ICAM scFv provide more industrially and clinically suitable biotherapeutics. Molecular design of these constructs permits deletion of unnecessary parts of molecules or insertion of point mutations endowing products with novel favorable pharmacokinetics and/or functional features. Clinical testing of antioxidant and antithrombotic biotherapeutics targeted to endothelial cells has been recently conducted and demonstrated promising levels of safety and efficacy. It is tempting to hope that in the next decade targeted interventions into endothelial cells will be translated from initial successes reported in laboratory animals to medical practice.

Acknowledgments

This work was supported by National Institutes of Health grants HL073940, HL091950, HL087036, and HL079063 to V.M.

References

1. Muzykantov VR. Biomedical aspects of targeted delivery of drugs to pulmonary endothelium. Expert Opin Drug Deliv. 2005; 2(5):909–926. [PubMed: 16296786]
2. Hajitou A, Trepel M, Lilley CE, et al. A hybrid vector for ligand-directed tumor targeting and molecular imaging. Cell. 2006; 125(2):385–398. [PubMed: 16630824]
3. Durr E, Yu J, Krasinska KM, et al. Direct proteomic mapping of the lung microvascular endothelial cell surface in vivo and in cell culture. Nat Biotechnol. 2004; 22(8):985–992. [PubMed: 15258593]
4. Oh P, Li Y, Yu J, et al. Subtractive proteomic mapping of the endothelial surface in lung and solid tumours for tissue-specific therapy. Nature. 2004; 429(6992):629–635. [PubMed: 15190345]
5. Rajotte D, Arap W, Hagedorn M, Koivunen E, Pasqualini R, Ruoslahti E. Molecular heterogeneity of the vascular endothelium revealed by in vivo phage display. J Clin Invest. 1998; 102(2):430–437. [PubMed: 9664085]
6. Ghitescu L, Jacobson BS, Crine P. A novel, 85 kDa endothelial antigen differentiates plasma membrane macro-domains in lung alveolar capillaries. Endothelium. 1999; 6(3):241–250. [PubMed: 10365775]
7. Murciano JC, Harshaw DW, Ghitescu L, Danilov SM, Muzykantov VR. Vascular immunotargeting to endothelial surface in a specific macrodomain in alveolar capillaries. Am J Respir Crit Care Med. 2001; 164(7):1295–1302. [PubMed: 11673225]
8. Oh P, Borgström P, Witkiewicz H, et al. Live dynamic imaging of caveolae pumping targeted antibody rapidly and specifically across endothelium in the lung. Nat Biotechnol. 2007; 25(3):327–337. [PubMed: 17334358]
9. Muzykantov VR, Puchnina EA, Atochina EN, et al. Endotoxin reduces specific pulmonary uptake of radio-labeled monoclonal antibody to angiotensin-converting enzyme. J Nucl Med. 1991; 32(3): 453–460. [PubMed: 1848608]
10. Danilov S, Atochina E, Hiemisch H, et al. Interaction of mAb to angiotensin-converting enzyme (ACE) with antigen in vitro and in vivo: antibody targeting to the lung induces ACE antigenic modulation. Int Immunol. 1994; 6(8):1153–1160. [PubMed: 7981144]
11. Danilov SM, Gavrilyuk VD, Franke FE, et al. Lung uptake of antibodies to endothelial antigens: key determinants of vascular immunotargeting. Am J Physiol Lung Cell Mol Physiol. 2001; 280(6):L1335–L1347. [PubMed: 11350815]
12. Heitsch H, Brovkovych S, Malinski T, Wiemer G. Angiotensin-(1-7)-stimulated nitric oxide and superoxide release from endothelial cells. Hypertension. 2001; 37(1):72–76. [PubMed: 11208759]

13. Muzykantov VR, Atochina EN, Kuo A, et al. Endothelial cells internalize monoclonal antibody to angiotensin-converting enzyme. Am J Physiol. 1996; 270(5 Pt 1):L704–L713. [PubMed: 8967503]
14. Danilov SM, Martynov AV, Klibanov AL, et al. Radio-immunoimaging of lung vessels: an approach using indium-111-labeled monoclonal antibody to angiotensin-converting enzyme. J Nucl Med. 1989; 30(10):1686–1692. [PubMed: 2552048]
15. Balyasnikova IV, Metzger R, Visintine DJ, et al. Selective rat lung endothelial targeting with a new set of monoclonal antibodies to angiotensin I-converting enzyme. Pulm Pharmacol Ther. 2005; 18(4):251–267. [PubMed: 15777608]
16. Balyasnikova IV, Sun ZL, Metzger R, et al. Monoclonal antibodies to native mouse angiotensin-converting enzyme (CD143): ACE expression quantification, lung endothelial cell targeting and gene delivery. Tissue Antigens. 2006; 67(1):10–29. [PubMed: 16451197]
17. Muzykantov, VR.; Danilov, SM. Targeting of radiolabeled monoclonal antibody against ACE to the pulmonary endothelium. In: Torchilin, V., editor. Targeted Delivery of Imaging Agents. Roca Baton, FL: CRC Press; 1995. p. 465-485.
18. Muzykantov VR, Atochina EN, Ischiropoulos H, Danilov SM, Fisher AB. Immunotargeting of antioxidant enzyme to the pulmonary endothelium. Proc Natl Acad Sci U S A. 1996; 93(11):5213–5218. [PubMed: 8643555]
19. Atochina EN, Balyasnikova IV, Danilov SM, Granger DN, Fisher AB, Muzykantov VR. Immunotargeting of catalase to ACE or ICAM-1 protects perfused rat lungs against oxidative stress. Am J Physiol. 1998; 275(4 Pt 1):L806–L817. [PubMed: 9755114]
20. Nowak K, Weih S, Metzger R, et al. Immunotargeting of catalase to lung endothelium via anti-angiotensin-converting enzyme antibodies attenuates ischemia-reperfusion injury of the lung in vivo. Am J Physiol Lung Cell Mol Physiol. 2007; 293(1):L162–L169. [PubMed: 17435080]
21. Watanabe K, Lam G, Keresztes RS, Jaffe EA. Lip-opolysaccharides decrease angiotensin converting enzyme activity expressed by cultured human endothelial cells. J Cell Physiol. 1992; 150(2):433–439. [PubMed: 1310327]
22. Atochina EN, Hiemisch HH, Muzykantov VR, Danilov SM. Systemic administration of platelet-activating factor in rat reduces specific pulmonary uptake of circulating monoclonal antibody to angiotensin-converting enzyme. Lung. 1992; 170(6):349–358. [PubMed: 1331624]
23. Scherpereel A, Wiewrodt R, Christofidou-Solomidou M, et al. Cell-selective intracellular delivery of a foreign enzyme to endothelium in vivo using vascular immunotargeting. FASEB J. 2001; 15(2):416–426. [PubMed: 11156957]
24. Danielyan K, Ding BS, Gottstein C, Cines DB, Muzykantov VR. Delivery of anti-platelet-endothelial cell adhesion molecule single-chain variable fragmenturokinase fusion protein to the cerebral vasculature lyses arterial clots and attenuates postischemic brain edema. J Pharmacol Exp Ther. 2007; 321(3):947–952. [PubMed: 17389242]
25. Garnacho C, Dhami R, Simone E, et al. Delivery of acid sphingomyelinase in normal and Niemann-Pick disease mice using intercellular adhesion molecule-1-targeted polymer nanocarriers. J Pharmacol Exp Ther. 2008; 325(2):400–408. [PubMed: 18287213]
26. Muro S, Dziubla T, Qiu W, et al. Endothelial targeting of high-affinity multivalent polymer nanocarriers directed to intercellular adhesion molecule 1. J Pharmacol Exp Ther. 2006; 317(3): 1161–1169. [PubMed: 16505161]
27. Muro S, Muzykantov VR. Targeting of antioxidant and antithrombotic drugs to endothelial cell adhesion molecules. Curr Pharm Des. 2005; 11(18):2383–2401. [PubMed: 16022673]
28. Nakada MT, Amin K, Christofidou-Solomidou M, et al. Antibodies against the first Ig-like domain of human platelet endothelial cell adhesion molecule-1 (PECAM-1) that inhibit PECAM-1-dependent homophilic adhesion block in vivo neutrophil recruitment. J Immunol. 2000; 164(1): 452–462. [PubMed: 10605042]
29. Murohara T, Delyani JA, Albelda SM, Lefer AM. Blockade of platelet endothelial cell adhesion molecule-1 protects against myocardial ischemia and reperfusion injury in cats. J Immunol. 1996; 156(9):3550–3557. [PubMed: 8617985]
30. Kumasaka T, Quinlan WM, Doyle NA, et al. Role of the intercellular adhesion molecule-1(ICAM-1) in endotoxin-induced pneumonia evaluated using ICAM-1 antisense

oligonucleotides, anti-ICAM-1 monoclonal antibodies, and ICAM-1 mutant mice. J Clin Invest. 1996; 97(10):2362–2369. [PubMed: 8636417]

31. Muro S, Wiewrodt R, Thomas A, et al. A novel endocytic pathway induced by clustering endothelial ICAM-1 or PECAM-1. J Cell Sci. 2003; 116(Pt 8):1599–1609. [PubMed: 12640043]
32. Muzykantov VR, Christofidou-Solomidou M, Balyasnikova I, et al. Streptavidin facilitates internalization and pulmonary targeting of an anti-endothelial cell antibody (platelet-endothelial cell adhesion molecule 1): a strategy for vascular immunotargeting of drugs. Proc Natl Acad Sci U S A. 1999; 96(5):2379–2384. [PubMed: 10051650]
33. Albelda SM. Endothelial and epithelial cell adhesion molecules. Am J Respir Cell Mol Biol. 1991; 4(3):195–203. [PubMed: 2001288]
34. Wiewrodt R, Thomas AP, Cipelletti L, et al. Size-dependent intracellular immunotargeting of therapeutic cargoes into endothelial cells. Blood. 2002; 99(3):912–922. [PubMed: 11806994]
35. Christofidou-Solomidou M, Pietra GG, Solomides CC, et al. Immunotargeting of glucose oxidase to endothelium in vivo causes oxidative vascular injury in the lungs. Am J Physiol Lung Cell Mol Physiol. 2000; 278(4):L794–L805. [PubMed: 10749757]
36. Scherpereel A, Rome JJ, Wiewrodt R, et al. Platelet-endothelial cell adhesion molecule-1-directed immunotargeting to cardiopulmonary vasculature. J Pharmacol Exp Ther. 2002; 300(3):777–786. [PubMed: 11861781]
37. Li S, Tan Y, Viroonchatapan E, Pitt BR, Huang L. Targeted gene delivery to pulmonary endothelium by anti-PECAM antibody. Am J Physiol Lung Cell Mol Physiol. 2000; 278(3):L504–L511. [PubMed: 10710522]
38. Muro S, Cui X, Gajewski C, Murciano JC, Muzykantov VR, Koval M. Slow intracellular trafficking of catalase nano-particles targeted to ICAM-1 protects endothelial cells from oxidative stress. Am J Physiol Cell Physiol. 2003; 285(5):C1339–C1347. [PubMed: 12878488]
39. Almenar-Queralt A, Duperray A, Miles LA, Felez J, Altieri DC. Apical topography and modulation of ICAM-1 expression on activated endothelium. Am J Pathol. 1995; 147(5):1278–1288. [PubMed: 7485391]
40. Panés J, Perry MA, Anderson DC, et al. Regional differences in constitutive and induced ICAM-1 expression in vivo. Am J Physiol. 1995; 269(6 Pt 2):H1955–H1964. [PubMed: 8594904]
41. Murciano JC, Muro S, Koniaris L, et al. ICAM-directed vascular immunotargeting of antithrombotic agents to the endothelial luminal surface. Blood. 2003; 101(10):3977–3984. [PubMed: 12531816]
42. Beck-Schimmer B, Schimmer RC, Warner RL, et al. Expression of lung vascular and airway ICAM-1 after exposure to bacterial lipopolysaccharide. Am J Respir Cell Mol Biol. 1997; 17(3):344–352. [PubMed: 9308921]
43. Vaporciyan AA, Mulligan MS, Warren JS, Barton PA, Miyasaka M, Ward PA. Up-regulation of lung vascular ICAM-1 in rats is complement dependent. J Immunol. 1995; 155(3):1442–1449. [PubMed: 7636209]
44. Sasso DE, Gionfriddo MA, Thrall RS, Syrbu SI, Smilowitz HM, Weiner RE. Biodistribution of indium-111-labeled antibody directed against intercellular adhesion molecule-1. J Nucl Med. 1996; 37(4):656–661. [PubMed: 8691262]
45. Villanueva FS, Jankowski RJ, Klibanov S, et al. Micro-bubbles targeted to intercellular adhesion molecule-1 bind to activated coronary artery endothelial cells. Circulation. 1998; 98(1):1–5. [PubMed: 9665051]
46. Bloemen PG, Henricks PA, van Bloois L, et al. Adhesion molecules: a new target for immunoliposome-mediated drug delivery. FEBS Lett. 1995; 357(2):140–144. [PubMed: 7805880]
47. Kishimoto TK, Rothlein R. Integrins, ICAMs, and selectins: role and regulation of adhesion molecules in neutrophil recruitment to inflammatory sites. Adv Pharmacol. 1994; 25:117–169. [PubMed: 7515640]
48. Springer TA. Adhesion receptors of the immune system. Nature. 1990; 346(6283):425–434. [PubMed: 1974032]
49. Spragg DD, Alford DR, Greferath R, et al. Immunotargeting of liposomes to activated vascular endothelial cells: a strategy for site-selective delivery in the cardiovascular system. Proc Natl Acad Sci U S A. 1997; 94(16):8795–8800. [PubMed: 9238057]

50. Kiely JM, Cybulsky MI, Luscinskas FW, Gimbrone MA Jr. Immunoselective targeting of an anti-thrombin agent to the surface of cytokine-activated vascular endothelial cells. Arterioscler Thromb Vasc Biol. 1995; 15(8):1211–1218. [PubMed: 7542999]
51. Kuijpers TW, Raleigh M, Kavanagh T, et al. Cytokine-activated endothelial cells internalize E-selectin into a lysosomal compartment of vesiculotubular shape. A tubulin-driven process. J Immunol. 1994; 152(10):5060–5069. [PubMed: 7513727]
52. Straley KS, Green SA. Rapid transport of internalized P-selectin to late endosomes and the TGN: roles in regulating cell surface expression and recycling to secretory granules. J Cell Biol. 2000; 151(1):107–116. [PubMed: 11018057]
53. von Asmuth EJ, Smeets EF, Ginsel LA, Onderwater JJ, Leeuwenberg JF, Buurman WA. Evidence for endocytosis of E-selectin in human endothelial cells. Eur J Immunol. 1992; 22(10):2519–2526. [PubMed: 1382989]
54. Kessner S, Krause A, Rothe U, Bendas G. Investigation of the cellular uptake of E-Selectin-targeted immunoliposomes by activated human endothelial cells. Biochim Biophys Acta. 2001; 1514(2):177–190. [PubMed: 11557019]
55. Kok RJ, Everts M, Asgeirsdóttir SA, Meijer DK, Molema G. Cellular handling of a dexamethasone-anti-E-selectin immunoconjugate by activated endothelial cells: comparison with free dexamethasone. Pharm Res. 2002; 19(11):1730–1735. [PubMed: 12458680]
56. Harari OA, Wickham TJ, Stocker CJ, et al. Targeting an adenoviral gene vector to cytokine-activated vascular endothelium via E-selectin. Gene Ther. 1999; 6(5):801–807. [PubMed: 10505104]
57. Lindner JR, Song J, Christiansen J, Klibanov AL, Xu F, Ley K. Ultrasound assessment of inflammation and renal tissue injury with microbubbles targeted to P-selectin. Circulation. 2001; 104(17):2107–2112. [PubMed: 11673354]
58. Kelly KA, Allport JR, Tsourkas A, Shinde-Patil VR, Josephson L, Weissleder R. Detection of vascular adhesion molecule-1 expression using a novel multimodal nano-particle. Circ Res. 2005; 96(3):327–336. [PubMed: 15653572]
59. Keelan ET, Harrison AA, Chapman PT, Binns RM, Peters AM, Haskard DO. Imaging vascular endothelial activation: an approach using radiolabeled monoclonal antibodies against the endothelial cell adhesion molecule E-selectin. J Nucl Med. 1994; 35(2):276–281. [PubMed: 7507524]
60. Lindner, JR.; Klibanov, AL.; Ley, K. Targeting inflammation. In: Muzykantov, VR.; Torchilin, VP., editors. Biomedical Aspects of Drug Targeting. Boston, MA: Kluwer Academic Publications; 2003. p. 149-172.
61. Bonaventura J, Gow A. NO and superoxide: opposite ends of the seesaw in cardiac contractility. Proc Natl Acad Sci U S A. 2004; 101(47):16403–16404. [PubMed: 15546989]
62. Christofidou-Solomidou M, Muzykantov VR. Antioxidant strategies in respiratory medicine. Treat Respir Med. 2006; 5(1):47–78. [PubMed: 16409015]
63. Cai H, Griendling KK, Harrison DG. The vascular NAD(P)H oxidases as therapeutic targets in cardiovascular diseases. Trends Pharmacol Sci. 2003; 24(9):471–478. [PubMed: 12967772]
64. McCord JM. Superoxide dismutase in aging and disease: an overview. Methods Enzymol. 2002; 349:331–341. [PubMed: 11912924]
65. Kinnula VL, Crapo JD. Superoxide dismutases in the lung and human lung diseases. Am J Respir Crit Care Med. 2003; 167(12):1600–1619. [PubMed: 12796054]
66. Beckman JS, Minor RL Jr, White CW, Repine JE, Rosen GM, Freeman BA. Superoxide dismutase and catalase conjugated to polyethylene glycol increases endothelial enzyme activity and oxidant resistance. J Biol Chem. 1988; 263(14):6884–6892. [PubMed: 3129432]
67. Giri SN, Misra HP. Fate of superoxide dismutase in mice following oral route of administration. Med Biol. 1984; 62(5):285–289. [PubMed: 6530902]
68. Corvo ML, Boerman OC, Oyen WJ, et al. Intravenous administration of superoxide dismutase entrapped in long circulating liposomes. II. In vivo fate in a rat model of adjuvant arthritis. Biochim Biophys Acta. 1999; 1419(2):325–334. [PubMed: 10407083]

69. Fujita T, Nishikawa M, Tamaki C, Takakura Y, Hashida M, Sezaki H. Targeted delivery of human recombinant superoxide dismutase by chemical modification with mono-and polysaccharide derivatives. J Pharmacol Exp Ther. 1992; 263(3):971–978. [PubMed: 1469654]
70. Freeman BA, Young SL, Crapo JD. Liposome-mediated augmentation of superoxide dismutase in endothelial cells prevents oxygen injury. J Biol Chem. 1983; 258(20):12534–12542. [PubMed: 6688807]
71. Day BJ, Shawen S, Liochev SI, Crapo JD. A metal-loporphyrin superoxide dismutase mimetic protects against paraquat-induced endothelial cell injury, in vitro. J Pharmacol Exp Ther. 1995; 275(3):1227–1232. [PubMed: 8531085]
72. Duann P, Datta PK, Pan C, Blumberg JB, Sharma M, Lianos EA. Superoxide dismutase mimetic preserves the glomerular capillary permeability barrier to protein. J Pharmacol Exp Ther. 2006; 316(3):1249–1254. [PubMed: 16303918]
73. Tamura Y, Chi LG, Driscoll EM Jr, et al. Superoxide dismutase conjugated to polyethylene glycol provides sustained protection against myocardial ischemia/reperfusion injury in canine heart. Circ Res. 1988; 63(5):944–959. [PubMed: 3180357]
74. Igarashi R, Hoshino J, Ochiai A, Morizawa Y, Mizushima Y. Lecithinized superoxide dismutase enhances its pharmacologic potency by increasing its cell membrane affinity. J Pharmacol Exp Ther. 1994; 271(3):1672–1677. [PubMed: 7996483]
75. Koo DD, Welsh KI, West NE, et al. Endothelial cell protection against ischemia/reperfusion injury by lecithinized superoxide dismutase. Kidney Int. 2001; 60(2):786–796. [PubMed: 11473663]
76. Ishihara T, Tanaka K, Tasaka Y, et al. Therapeutic effect of lecithinized superoxide dismutase against colitis. J Pharmacol Exp Ther. 2009; 328(1):152–164. [PubMed: 18927353]
77. Lee S, Murthy N. Targeted delivery of catalase and superoxide dismutase to macrophages using folate. Biochem Biophys Res Commun. 2007; 360(1):275–279. [PubMed: 17586472]
78. Gow A, Ischiropoulos H. Super-SOD: superoxide dismutase chimera fights off inflammation. Am J Physiol Lung Cell Mol Physiol. 2003; 284(6):L915–L916. [PubMed: 12736187]
79. Bonder CS, Knight D, Hernandez-Saavedra D, McCord JM, Kubes P. Chimeric SOD2/3 inhibits at the endothelial-neutrophil interface to limit vascular dysfunction in ischemia-reperfusion. Am J Physiol Gastrointest Liver Physiol. 2004; 287(3):G676–G684. [PubMed: 15087276]
80. Gao B, Flores SC, Leff JA, Bose SK, McCord JM. Synthesis and anti-inflammatory activity of a chimeric recombinant superoxide dismutase: SOD2/3. Am J Physiol Lung Cell Mol Physiol. 2003; 284(6):L917–L925. [PubMed: 12736188]
81. Grey M, Yainoy S, Prachayasittikul V, Bülow L. A super-oxide dismutase-human hemoglobin fusion protein showing enhanced antioxidative properties. FEBS J. 2009; 276(21):6195–6203. [PubMed: 19788422]
82. Lu M, Gong X, Lu Y, Guo J, Wang C, Pan Y. Molecular cloning and functional characterization of a cell-permeable superoxide dismutase targeted to lung adenocarcinoma cells. Inhibition cell proliferation through the Akt/p27kip1 pathway. J Biol Chem. 2006; 281(19):13620–13627. [PubMed: 16551617]
83. Jiang J, Kurnikov I, Belikova NA, et al. Structural requirements for optimized delivery, inhibition of oxidative stress, and antiapoptotic activity of targeted nitroxides. J Pharmacol Exp Ther. 2007; 320(3):1050–1060. [PubMed: 17179468]
84. Nishikawa M, Hashida M, Takakura Y. Catalase delivery for inhibiting ROS-mediated tissue injury and tumor metastasis. Adv Drug Deliv Rev. 2009; 61(4):319–326. [PubMed: 19385054]
85. Muzykantov VR, Christofidou-Solomidou M, Balyasnikova I, et al. Streptavidin facilitates internalization and pulmonary targeting of an anti-endothelial cell antibody (platelet-endothelial cell adhesion molecule 1): a strategy for vascular immunotargeting of drugs. Proc Natl Acad Sci U S A. 1999; 96(5):2379–2384. [PubMed: 10051650]
86. Christofidou-Solomidou M, Scherpereel A, Wiewrodt R, et al. PECAM-directed delivery of catalase to endothelium protects against pulmonary vascular oxidative stress. Am J Physiol Lung Cell Mol Physiol. 2003; 285(2):L283–L292. [PubMed: 12851209]
87. Shuvaev VV, Dziubla T, Wiewrodt R, Muzykantov VR. Streptavidin-biotin crosslinking of therapeutic enzymes with carrier antibodies: nanoconjugates for protection against endothelial oxidative stress. Methods Mol Biol. 2004; 283:3–19. [PubMed: 15197299]

88. Shuvaev VV, Christofidou-Solomidou M, Bhora F, et al. Targeted detoxification of selected reactive oxygen species in the vascular endothelium. J Pharmacol Exp Ther. 2009; 331(2):404–411. [PubMed: 19692634]

89. Kozower BD, Christofidou-Solomidou M, Sweitzer TD, et al. Immunotargeting of catalase to the pulmonary endothelium alleviates oxidative stress and reduces acute lung transplantation injury. Nat Biotechnol. 2003; 21(4):392–398. [PubMed: 12652312]

90. Shuvaev VV, Tliba S, Nakada M, Albelda SM, Muzykantov VR. Platelet-endothelial cell adhesion molecule-1-directed endothelial targeting of superoxide dismutase alleviates oxidative stress caused by either extracellular or intracellular superoxide. J Pharmacol Exp Ther. 2007; 323(2): 450–457. [PubMed: 17712041]

91. Muro S, Mateescu M, Gajewski C, Robinson M, Muzykantov VR, Koval M. Control of intracellular trafficking of ICAM-1-targeted nanocarriers by endothelial Na+/H+ exchanger proteins. Am J Physiol Lung Cell Mol Physiol. 2006; 290(5):L809–L817. [PubMed: 16299052]

92. Muro S, Gajewski C, Koval M, Muzykantov VR. ICAM-1 recycling in endothelial cells: a novel pathway for sustained intracellular delivery and prolonged effects of drugs. Blood. 2005; 105(2): 650–658. [PubMed: 15367437]

93. Dziubla TD, Karim A, Muzykantov VR. Polymer nano-carriers protecting active enzyme cargo against proteolysis. J Control Release. 2005; 102(2):427–439. [PubMed: 15653162]

94. Simone EA, Dziubla TD, Arguiri E, et al. Loading PEG-catalase into filamentous and spherical polymer nanocarriers. Pharm Res. 2009; 26(1):250–260. [PubMed: 18956141]

95. Dziubla TD, Shuvaev VV, Hong NK, et al. Endothelial targeting of semi-permeable polymer nanocarriers for enzyme therapies. Biomaterials. 2008; 29(2):215–227. [PubMed: 17950837]

96. Esmon CT. Inflammation and thrombosis. J Thromb Haemost. 2003; 1(7):1343–1348. [PubMed: 12871267]

97. Bode C, Meinhardt G, Runge MS, et al. Platelet-targeted fibrinolysis enhances clot lysis and inhibits platelet aggregation. Circulation. 1991; 84:805–813. [PubMed: 1860223]

98. Runge MS, Quertermous T, Zavodny PJ, et al. A recombinant chimeric plasminogen activator with high affinity for fibrin has increased thrombolytic potency in vitro and in vivo. Proc Natl Acad Sci U S A. 1991; 88(22):10337–10341. [PubMed: 1946453]

99. Waugh JM, Kattash M, Li J, et al. Gene therapy to promote thromboresistance: local overexpression of tissue plasminogen activator to prevent arterial thrombosis in an in vivo rabbit model. Proc Natl Acad Sci U S A. 1999; 96(3):1065–1070. [PubMed: 9927694]

100. Topol EJ, Byzova TV, Plow EF. Platelet GPIIb-IIIa blockers. Lancet. 1999; 353(9148):227–231. [PubMed: 9923894]

101. Muzykantov VR, Barnathan ES, Atochina EN, Kuo A, Danilov SM, Fisher AB. Targeting of antibody-conjugated plasminogen activators to the pulmonary vasculature. J Pharmacol Exp Ther. 1996; 279(2):1026–1034. [PubMed: 8930213]

102. Murciano JC, Harshaw DW, Ghitescu L, Danilov SM, Muzykantov VR. Vascular immunotargeting to endothelial surface in a specific macrodomain in alveolar capillaries. Am J Respir Crit Care Med. 2001; 164(7):1295–1302. [PubMed: 11673225]

103. Ding BS, Zhou YJ, Chen XY, et al. Lung endothelium targeting for pulmonary embolism thrombolysis. Circulation. 2003; 108(23):2892–2898. [PubMed: 14610017]

104. Muro S, Cui X, Gajewski C, Murciano JC, Muzykantov VR, Koval M. Slow intracellular trafficking of catalase nano-particles targeted to ICAM-1 protects endothelial cells from oxidative stress. Am J Physiol Cell Physiol. 2003; 285(5):C1339–C1347. [PubMed: 12878488]

105. Idell S. Coagulation, fibrinolysis, and fibrin deposition in acute lung injury. Crit Care Med. 2003; 31(4, suppl):S213–S220. [PubMed: 12682443]

106. Levi M, Schultz M. The inflammation-coagulation axis as an important intermediate pathway in acute lung injury. Crit Care. 2008; 12(2):144. [PubMed: 18466641]

107. Wu SQ, Aird WC. Thrombin, TNF-alpha, and LPS exert overlapping but nonidentical effects on gene expression in endothelial cells and vascular smooth muscle cells. Am J Physiol Heart Circ Physiol. 2005; 289:H873–H885. [PubMed: 15833800]

108. Ding BS, Gottstein C, Grunow A, et al. Endothelial targeting of a recombinant construct fusing a PECAM-1 single-chain variable antibody fragment (scFv) with prourokinase facilitates

prophylactic thrombolysis in the pulmonary vasculature. Blood. 2005; 106(13):4191–4198. [PubMed: 16144802]

109. Stump DC, Lijnen HR, Collen D. Purification and characterization of a novel low molecular weight form of single-chain urokinase-type plasminogen activator. J Biol Chem. 1986; 261(36): 17120–17126. [PubMed: 3097021]
110. Blasi F, Carmeliet P. uPAR: a versatile signalling orchestrator. Nat Rev Mol Cell Biol. 2002; 3(12):932–943. [PubMed: 12461559]
111. Liu JN, Gurewich V. Fragment E-2 from fibrin substantially enhances prourokinase-induced Glu-plasminogen activation. A kinetic study using the plasmin-resistant mutant prourokinase Ala-158-rpro-UK. Biochemistry. 1992; 31(27):6311–6317. [PubMed: 1385727]
112. Ichinose A, Fujikawa K, Suyama T. The activation of prourokinase by plasma kallikrein and its inactivation by thrombin. J Biol Chem. 1986; 261(8):3486–3489. [PubMed: 3081506]
113. Yang WP, Goldstein J, Procyk R, Matsueda GR, Shaw SY. Design and evaluation of a thrombin-activable plasminogen activator. Biochemistry. 1994; 33:606–612. [PubMed: 8286392]
114. Ding BS, Hong N, Murciano JC, et al. Prophylactic thrombolysis by thrombin-activated latent prourokinase targeted to PECAM-1 in the pulmonary vasculature. Blood. 2008; 111(4):1999–2006. [PubMed: 18045968]
115. Ding BS, Hong NK, Christofidou-Solomidou M, et al. Anchoring fusion thrombomodulin to the endothelial lumen protects against injury-induced lung thrombosis and inflammation. Am J Respir Crit Care Med. 2009; 180(3):247–256. [PubMed: 19342415]
116. Wang YX, Wu C, Vincelette J, et al. Amplified anticoagulant activity of tissue factor-targeted thrombomodulin: in-vivo validation of a tissue factor-neutralizing antibody fused to soluble thrombomodulin. Thromb Haemost. 2006; 96(3):317–324. [PubMed: 16953273]

Table 1

Candidate Targets for Endothelial Drug Delivery

Target	Function and Localization	Targeting Advantages	Potential Problems
ACE	Peptidase, converts Ang I into Ang II and cleaves bradykinin, ACE enriched in the lung capillaries	Selective targeting to lung EC, intracellular delivery, vasodilating and anti-inflammatory effects of ACE inhibition	Inflammation suppresses targeting; ACE inhibition may be dangerous
PECAM-1	Facilitates transmigration of leukocytes, stably expressed in EC borders	Intracellular delivery of anti-PECAM conjugate may also suppress inflammation	PECAM signaling and side effects are not understood
ICAM-1	Mediates leukocyte adhesion to EC, stably expressed and upregulated by pathologies	Similar to PECAM, but inflammation enhances targeting	Similar to PECAM
E-selectin	Supports leukocyte adhesion, expressed only on altered EC	Intracellular targeting to EC in inflammation	Targeting is transient and not robust; surface targeting is not effective due to endocytosis
P-selectin	Similar to E-selectin	Similar to E-selectin	Similar to above, platelets also bind P-selectin ligands
gp90	Function unknown; localized in EC caveoli	Transendothelial targeting in rat lungs	Human analog and side effects are not known
Gp85	Function unknown; EC avesicular zone in alveolar capillaries	Targeting to the EC surface in rat lungs	Similar to above
gp60	Albumin-binding protein in EC caveoli	Transendothelial targeting	Unclear side effects and specificity of targeting
APN	Digestion of peptides by proteolysis	Upregulated in tumor vasculature	Expression in other cell types
APP	Cleavage of bradykinin and substance P	Localized in the caveoli, enriched in lungs, and offers very effective and fast endothelial targeting	Inhibition of APP may cause side effects including edema

ACE, angiotensin-converting enzyme; EC, endothelial cells; PECAM, platelet-endothelial adhesion molecule; ICAM, intercellular adhesion molecule; gp, glycoproteins; APN, aminopeptidase N; APP, aminopeptidase P.

Table 2

Antioxidant and Antithrombotic Interventions

Cargo	Target	Disease	Effect
Antioxidant Interventions			
PC-SOD	EC membrane	Myocardial ischemia, colitis, burn trauma, etc.	Attenuation of tissue injury
Chimera SOD2/3	EC HSPGs	I/R	Attenuation of tissue injury
Antibody/SOD conjugate	PECAM-1	Hypertension	Vasoreactivity normalization, BH_4 protection against oxidation
Antibody/catalase conjugate	PECAM-1	I/R	↓ Alveolar edema and attenuation of arterial blood oxygenation
Antithrombotic Interventions			
tPA	ICAM-1	CVD	↑ Rate of fibrinolysis
uPA	PECAM-1	CVD	↑ Rate of fibrinolysis
uPA-T	PECAM-1	CVD	↑ ↑ Rate of fibrinolysis
TM	PECAM-1	ALI	↓ Lung thrombosis and inflammation

PC, phosphatidylcholine; SOD, superoxide dismutase; EC, endothelial cells; HSPG, heparan sulfate proteoglycan; I/R, ischemia/reperfusion; PECAM-1, pulmonary endothelial cell adhesion molecule-1; BH_4, tetrahydrobiopterin; tPA, tissue plasminogen activator; ICAM-1, intercellular adhesion molecule-1; CVD, cardiovascular disease; uPA, urokinase plasminogen activator; uPA-T, urokinase plasminogen activator–thrombin; TM, thrombomodulin; ALI, acute lung injury.