Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Differential involvement of sulodexide in lipopolysaccharide- and pneumolysin-induced lung endothelial barrier dysfunction in vitro and in vivo

Latika Jaiswal
Augusta University
Peter Biggs
Augusta University
Mary Cherian-shaw
Augusta University
Liselle Simon
Augusta University
Shweta R. Patil
Augusta University
Basmah Mohamed
Augusta University
Rahul Shivaji Patil
Augusta University
Rudolf Lucas
Augusta University
Anita Kovacs-Kasa
Augusta University
Alexander Verin
averin@augusta.edu

Augusta University

Research Article

Keywords: Acute lung injury, sulodexide, pneumolysin, lipopolysaccharide, glycocalyx, endothelial barrier integrity

Posted Date: November 4th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11116820/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are defined by impairment of the pulmonary endothelial barrier, resulting in vascular permeability and pulmonary edema. Toxins such as lipopolysaccharide (LPS) from Gram positive bacteria and pneumolysin (PLY) from Gram negative bacteria contribute to endothelial injury by impairing the glycocalyx and intercellular connections. This study investigated the protective effects of sulodexide (SDX), a glycocalyx-preserving agent, against LPS- and PLY-induced endothelial barrier dysfunction in human lung microvascular endothelial cells (HLMVECs) and murine ALI models. Endothelial barrier integrity was evaluated using transendothelial electrical resistance (TER), junctional integrity by immunofluorescence staining for VE-cadherin and F-actin, and lung vascular leak by Evans Blue Dye (EBD) assay, and protein content by bronchoalveolar lavage fluid (BALF). Pretreatment with SDX mitigated PLY's effects on TER, junctional integrity, and *in vivo* vascular leak, and post-treatment with SDX restored TER after PLY insult. SDX's effects on LPS-induced TER decline and lung permeability, while significant, were less pronounced. These findings suggest SDX protects lung endothelial barrier integrity *in vitro* and *in vivo* in a toxin-specific manner, with more evident protection against pore-forming toxins like PLY than against receptor-mediated toxins like LPS. SDX may represent a therapeutic strategy for preventing vascular leakage and lung injury in Gram-positive bacterial infections and inflammatory lung disease.

Introduction

Acute lung injury, along with its more severe form, acute respiratory distress syndrome, endure as one of the leading contributors to illness and death in the US *[1,2] [3]*. ALI/ARDS are characterized by diffuse pulmonary endothelial barrier disruption, which drives increased vascular permeability, protein-rich alveolar edema, impaired gas exchange, and severe hypoxemia, contributing to high mortality despite improvements in supportive care [*4,5*]; [*1,6*]. The pulmonary endothelium forms the primary interface between circulating inflammatory mediators and the lung parenchyma, and its integrity plays a critical role in determining disease severity and clinical outcome [*4,7*]. Toxins derived from Gram positive G + and G- bacteria are potential triggers of ALI *[8*]. They directly or indirectly damage the endothelial glycocalyx and thereby compromise vascular integrity [*9]*. We hypothesized that preservation of the endothelial glycocalyx with sulodexide can protect the pulmonary endothelial barrier against bacterial toxin–induced injury and that its efficacy depends on the mechanism of endothelial disruption.

Endothelial barrier integrity is maintained through coordinated interactions among inter endothelial junctions, the actin cytoskeleton and the endothelial glycocalyx *[10]*; [*11*]. The glycocalyx is a dynamic, carbohydrate-rich layer composed of proteoglycans, glycoproteins, and glycosaminoglycans that covers the luminal surface of endothelial cells and regulates vascular homeostasis [*12,7,10*]. It restricts plasma protein leakage, maintains oncotic balance in accordance with the revised Starling principle, senses shear stress, and limits inappropriate endothelial activation *[12] [13]*. Heparan sulfate proteoglycans, particularly syndecan-1, represent major structural components essential for glycocalyx stability and barrier function *[7,14]*.

During ALI/ARDS, the glycocalyx is rapidly degraded through enzymatic and inflammatory processes. Heparinase-mediated shedding of heparan sulfate chains and shedding of syndecan-1 ectodomains result in progressive glycocalyx loss [*12,14*]. This degradation is associated with disruption of inter endothelial junctions, including reduced expression and disorganization of VE-cadherin and zonula occludens-1, driving increased permeability across the paracellular space [*14*]. Importantly, circulating markers of glycocalyx degradation, including heparan sulfate and syndecan-1, are increased in patients with sepsis and ARDS and correlate with disease severity and mortality, including pediatric ARDS *[14] [15]*. These findings highlight the glycocalyx as both a biomarker and a driver of endothelial dysfunction.

ALI is commonly triggered by bacterial pneumonia and sepsis induced by both Gram-positive (G+) and Gram-negative (G-) organisms; however, G + and G- bacterial products disrupt endothelial integrity through distinct mechanisms. LPS, the prototypical G+ endotoxin, signals through a receptor-mediated pathway involving Toll-like receptor 4 (TLR4) and CD14 [*16*]. This activates NF-κB–mediated transcription of inflammatory genes and downstream signaling cascades involving RhoA/Rho-kinase activation, GEF-H1–mediated microtubule destabilization, and TRPC6-dependent calcium influx, ultimately resulting in cytoskeletal contraction and inter endothelial gap formation *[5] [16] [17]*.

In contrast, the G+ toxin and key virulence factor of *Streptococcus pneumoniae*, PLY, induces endothelial injury through direct membrane disruption rather than receptor activation. PLY is a cholesterol-dependent pore-forming cytolysin that inserts into the plasma membrane and forms transmembrane pores. Sub-lytic pore formation leads to excessive calcium influx, activation of protein kinase C-α, imbalance of RhoA/Rac1 signaling, increased phosphorylation of myosin light chain, and cytoskeletal destabilization, culminating in VE-cadherin disassembly and barrier failure *[18] [19]*. Clinically, this mechanism contributes to persistent pulmonary edema even after bacterial clearance, highlighting the direct membrane-toxic nature of PLY.

Considering the central role played by the endothelial glycocalyx in barrier regulation, therapeutic strategies aimed at its restoration have gained increasing attention. Sulodexide, a glycosaminoglycan-based compound made up of roughly 80% heparin-like/heparan sulfate fractions and 20% dermatan sulfate, represents a glycocalyx-targeted therapy *[20] [21]*. SDX restores glycocalyx integrity by supplying glycosaminoglycan precursors and inhibiting heparanase activity, thereby limiting glycocalyx degradation *[16] [20]*.

In addition to structural effects, SDX exerts anti-inflammatory actions by suppressing NF-κB signaling and reducing cytokine and chemokine release from endothelial cells and macrophages *[22] [23]*. It also inhibits PLA2/COX-2/VEGF signaling pathways and endothelial activation *[24*]. Experimentally and clinically, SDX has been demonstrated to preserve glycocalyx integrity, decrease vascular permeability, and improve endothelial function in diabetes and sepsis *[20] [16] [14]*.

Despite these findings, it remains unclear whether glycocalyx-targeted therapy provides equivalent protection against endothelial injury induced through distinct mechanisms. Specifically, whether SDX confers similar protection against receptor-mediated inflammatory injury (LPS) and direct membrane-

disruptive injury (PLY) is unknown. Therefore, we assessed the impact of SDX on pulmonary endothelial barrier integrity in response to LPS and PLY using human lung microvascular endothelial cells, as well as *in-vivo* murine models of ALI. Our data suggested that SDX conferred greater protection against PLY-induced barrier dysfunction than LPS-induced injury, likely due to differences in the underlying mechanisms of endothelial disruption.

Methodology

Materials

Sulodexide was kindly provided by ALFA WASSERMANN S.p.A., Bologna, Italy

Pneumolysin toxin was kindly provided by Dr. Chakraborty (Justus Liebig University Giessen, Germany). It was purified from a recombinant strain *Listeria innocua* 6a expressing PLY in his laboratory *[18]*. The PLY preparation used in this study exhibited a specific activity of 1.25×10^7 hemolytic units/mg.hu.

Lipopolysaccharide obtained from Escherichia coli O111:B4 purified by phenol extraction, endotoxin level 3,000,0000 endotoxin units/mg was purchased from Sigma-Aldrich (Saint Lois, MO, USA).

All other chemicals were purchased from Sigma-Aldrich (St. Louis), unless otherwise indicated

Cell Culture

Human Lung Microvascular Endothelial Cells obtained from Lonza Bioscience (Allandale, NJ, USA) were cultured in endothelial growth medium (EBM-2, Lonza and HPMEC-c Lot number: 1 x 480Z008.1 500,000) under standard conditions (37°C, 5% CO_2, humidified atmosphere). Cells were thawed and seeded at a density of 2,500 viable cells/cm² in T25 flasks. Media was changed at 24 h after seeding and later every 48 h thereafter. Cells were sub-cultured at around 85% confluency using Trypsin/EDTA and re-seeded into 6-well plates at a density of 0.3×10^6cells/well.

Transendothelial Electrical Resistance (TER)

Endothelial barrier integrity was reviewed in real time by measuring TER, an inverse EC permeability index [*25*]. TER was assessed using the ECIS-Zeta system (Electric Cell-substrate Impedance Sensing; Applied Biophysics, Troy, NY, USA). HLMVECs were plated at a density of 6×10^4 cells per well onto electrode-embedded 8W10E ECIS arrays and cultured until reaching confluence. Baseline resistance values were recorded prior to treatment. Confluent monolayers of cells received one of the following treatments: untreated, PLY (150 ng/ml) or 100 e.u./mL of LPS, SDX (100 µg/ml) from few minutes to an hour prior/after.

Changes in TER following PLY and LPS stimulation were monitored at indicated time points to evaluate endothelial permeability and barrier dysfunction.

Immunofluorescent staining

HLMVECs were cultured on sterile glass coverslips until reaching confluence. After exposure to LPS or PLY, cells were rinsed with phosphate-buffered saline (PBS) and fixed using 4% paraformaldehyde for 15 minutes at room temperature (RT). Membrane permeabilization was achieved with 0.1% Triton X-100 for 10 minutes, followed by a 1-hour blocking step at RT using 5% bovine serum albumin (BSA). Cells were then incubated overnight at 4°C with a primary antibody targeting VE-cadherin, diluted in blocking buffer. After washing with PBS, cells were exposed to a fluorescently labeled secondary antibody (Alexa Fluor 488-conjugated goat anti-rabbit IgG, A11034, 1:200, Invitrogen, USA) for 1 hour at RT, protected from light. Nuclear staining was performed using DAPI, while actin filaments were visualized with Texas red-conjugated phalloidin (T7471, 1:200, Invitrogen, USA) applied for 1 hour at RT.

Slides were prepared by mounting coverslips with antifade mounting medium, and imaging was performed on a fluorescence microscope (Axiolab; Carl Zeiss, using Zeiss Axiolab software). Endothelial barrier disruption following PLY and LPS exposure was evaluated by examining VE-cadherin distribution patterns and the integrity of cell-cell junctions.

Animal experiments

Male C57BL/6 mice (19–25 g) were obtained from Envigo RMS, Inc. (Indianapolis, IN). Both LPS and PLY were diluted in saline (LPS-1mg/ml & PLY- 4µg/ml) before dosing. Prior to any injection, animals were anesthetized using a combination of ketamine (150 mg/kg body weight) and acetopromazine (15 mg/kg body weight). Intratracheal (IT) delivery of both toxins, LPS (1 mg/kg body weight) and PLY (2.7 µg/kg body weight) was performed using a 20-gauge catheter. One hour prior to toxin administration, mice received either SDX (10 mg/kg body weight) or 0.9% saline via tail vein injection. To evaluate vascular permeability, Evans blue dye-albumin (30 mg/kg body weight) was administered by tail vein injection 2 hours before the study's endpoint, allowing assessment of extravasation. For protein quantification and cell counting, bronchoalveolar lavage (BAL) was performed by lavaging the lungs with 1 mL of 10% HBSS to obtain the bronchoalveolar lavage fluid (BALF). All procedures adhered to NIH guidelines and received prior approval from the Augusta University Institutional Animal Care and Use Committee.

Measurement of lung permeability (Evans Blue Dye (EBD) albumin extravasation) into lung tissue.

Following anesthesia, EBD-albumin was injected into the tail vein 2hrs before the termination of the experiment to assess vascular leak *[26*]. For assessment of Evans blue dye extravasation, the left lungs were weighed and homogenized, incubated with formamide (18 hours at 60° C) and spun at 5000g for 30 minutes. The optical density of supernatant was measured spectrophotometrically at 620 nm and

750 nm. EBD concentration in lung homogenate was then determined against standard curve (mgs of Evans Blue dye per gram lung) and expressed relative to lung weight.

Bronchoalveolar lavage fluid (BALF) for protein estimation

To separate cells from the fluid fraction, BALF samples were first centrifuged at 500 × g for 15 min at 4°C first and then supernatant was then centrifuged at 2500 × g for 10 min at 4°C to eliminate any remaining debris, yielding clarified BALF suitable for subsequent total protein quantification. The cell pellet generated after the initial centrifugation was treated with ACK lysis buffer to eliminate red blood cells, with gentle mixing maintained throughout a 15-minute incubation period. Cells were then pelleted again via centrifugation (500 × g, 15 minutes, 4°C), after which the cells were resuspended in Hank's solution and examined for total number white blood cells using a hemocytometer.

Results

Sulodexide modulates changes in TER and cytoskeletal/junctional remodeling induced by PLY and LPS in HLMVECs

To determine whether SDX protects endothelial barrier function against G+ bacterial toxin induced EC injury, we first investigated the effect of SDX on microvascular endothelial permeability using ECIS measurements. HLMVEC monolayers were challenged with SDX either before or after PLY treatment (Fig. 1. A, B). PLY markedly reduced transendothelial resistance, indicating disruption of endothelial barrier integrity. These findings are consistent with previous reports on the role of PLY in EC barrier compromise *[27] [28]* and ALI induced by pneumococcal pneumonia [*29*]. Importantly that SDX significantly attenuated the PLY-induced decrease in TER when administered either as a pretreatment or following PLY exposure, demonstrating both protective and restorative effects on endothelial barrier function in PLY EC injury model.

To determine whether SDX protects endothelial barrier function against G- (LPS)-induced inflammatory injury, HLMVEC monolayers were initially pretreated with SDX prior to LPS challenge (Fig. 1C). Consistently with our previous reports [*30–32*], LPS markedly decreased TER, indicating disruption of endothelial barrier integrity. Pretreatment with SDX significantly attenuated the LPS-induced reduction in TER, demonstrating a protective effect on endothelial barrier function. However, post-treatment of SDX exposure did not affect the LPS induced decrease in TER, indicating that SDX is more effective when present before inflammatory injury occurs (Fig. 1D). Apparently, the effects of SDX on LPS-induced TER decline were less pronounced as compared to the effects on PLY-induced EC permeability suggesting different protective mechanisms.

Sulodexide preserves endothelial barrier integrity by preventing PLY- and LPS-induced cytoskeletal remodeling and by maintaining VE-cadherin expression at cell junctions

To further evaluate whether SDX protects pulmonary endothelial barrier integrity, immunofluorescence staining was performed to define changes in expression and subcellular distribution of the adherens junction protein VE-cadherin and the actin cytoskeleton (F-actin) in HLMVECs in response to PLY and LPS exposure (Fig. 2A, B). demonstrated that quiescent control cells exhibited continuous VE-cadherin staining along cell-cell borders together with well-organized cortical F-actin, indicating intact endothelial junctions and preserved barrier integrity *[33]*.

Exposure to PLY caused marked disruption of endothelial junctions, characterized by fragmented and discontinuous VE-cadherin staining, and prominent F-actin stress fiber formation suggesting the loss of EC monolayer integrity consistently with endothelial hyperpermeability (Fig. 2A). These observations are in agreement with previous studies demonstrating that PLY disrupts endothelial adherens junctions and promotes cytoskeletal remodeling, leading to barrier dysfunction *[34*].

Similarly, LPS exposure leads to marked disruption of endothelial junctions, characterized by fragmented and discontinuous VE-cadherin staining and prominent F-actin stress fiber formation indicating the loss of EC barrier integrity, which is consistent with endothelial hyperpermeability (TER decline, Fig. 2B). These observations are in agreement with previous studies demonstrating that LPS induces endothelial barrier dysfunction through adherens junction disruption and actin cytoskeletal remodeling *[35] [36]*.

Pretreatment with SDX markedly attenuates these toxin-induced alterations in endothelial morphology, as evidenced by preservation of continuous VE-cadherin localization at intercellular junctions, reduced F-actin stress fiber formation, and maintenance of endothelial monolayer continuity compared with treated cells. Collectively, these findings demonstrate that SDX protects against both PLY and LPS-induced endothelial barrier injury by stabilizing adherens junctions and limiting cytoskeletal remodeling, thereby preserving pulmonary endothelial barrier function.

Collectively, these findings demonstrate that SDX efficiently protects and restores EC barrier in PLY-induced (G+) lung EC injury model. However, the SDX protective effects against LPS (G-)-induced EC are less pronounced. SDX effects on EC barrier integrity are likely associated with maintaining EC adherens junctions (VE-cadherin) and limiting F-actin cytoskeletal rearrangement induced by bacterial toxins.

Sulodexide attenuates LPS- and PLY-induced pulmonary vascular permeability in vivo

To determine whether SDX protects against acute lung vascular barrier dysfunction *In-vivo*, mice received an intravenous injection of SDX 1 h prior to intratracheal administration of LPS or PLY. Pulmonary vascular permeability was assessed by Evans Blue dye extravasation.

Consistent with the previous reports [*37*,*30*,*38–40*], LPS and PLY challenge significantly increased Evans Blue dye accumulation in lung tissue compared with the vehicle (control) group, reflecting disruption of the pulmonary microvascular barrier *[41]*. Pretreatment with SDX significantly reduced Evans Blue dye extravasation in both the LPS- and PLY-treated groups, demonstrating that SDX efficiently protects pulmonary microvascular barrier integrity against edemagenic toxins, PLY and LPS (Fig. 3. A, B).

To further characterize the effect of SDX on the alveolar-capillary barrier in ALI, total protein concentration in BALF was measured as an indicator of protein extravasation into the lung airspaces. PLY administration significantly increased BALF protein concentration compared with vehicle (control) animals, consistent with increased alveolar-capillary permeability. Pretreatment with SDX significantly reduced PLY-induced protein extravasation into the lung airspace (Fig. 3. C). This observation is consistent with the previous findings demonstrating that therapies that stabilize endothelial junctions and the glycocalyx may be effective against PLY-induced lung injury *[34*].

In contrast, while LPS significantly increases BALF protein concentration compared with the vehicle (control) group, pretreatment with SDX does not significantly reduce LPS-induced BALF protein accumulation (Fig. 3. D). These findings suggest that while SDX effectively prevents LPS-induced vascular permeability within the pulmonary microvasculature, it does not significantly attenuate LPS-mediated protein extravasation into the alveolar airspace under the present experimental conditions. Protein accumulation in BALF is broader parameter of lung injury as compared to Evans Blue Dye flux and aside microvascular permeability it reflects damage of alveolar epithelium and protein release from inflammatory and lung epithelial cells [*41*]. It is possible that intravenous SDX administration is less effective against ALI induced by intratracheal LPS, known by severe epithelial damage and inflammation [*42,43*]. Collectively, these data demonstrate that prophylactic SDX administration provides robust protection against lung microvascular leakage induced by both LPS and PLY, whereas its protective effect on alveolar protein leakage is more pronounced following PLY challenge than LPS exposure.

Discussion

The present study demonstrates that LPS and PLY, the G- and G+ bacterial toxins, respectively, induce significant endothelial disruption of barrier function in HLMVECs and in mice whereas pretreatment with glycocalyx-stabilizing agent, SDX, efficiently preserves endothelial integrity and attenuates toxin-induced ALI. However, the magnitude of SDX-induced EC barrier protection is toxin-specific. To our knowledge, this is the first report directly comparing the effect of SDX on PLY and LPS-induced loss of EC barrier integrity in HLMVEC and in mice. These findings highlight the critical contribution of the endothelial glycocalyx to pulmonary vascular homeostasis and support glycocalyx-targeted therapy as a promising therapeutic strategy for the treating ALI/ARDS.

The pulmonary endothelial barrier is a key regulator of vascular permeability and maintaining fluid balance within the lung. Disruption of this barrier integrity is a hallmark of ALI and ARDS and results in pulmonary edema, inflammatory cell infiltration, and compromised gas exchange *[44]*. In the present study, both LPS and PLY markedly reduce transendothelial electrical resistance of HLMVEC monolayers, disrupt EC junctions and increase lung vascular permeability in mice. These findings are consistent with numerous previous reports demonstrating that bacterial toxins are potent mediators of endothelial injury and vascular leak *[8]*.

LPS, a principal constituent of G- bacterial membranes, activates TLR4-dependent signaling pathways that trigger inflammatory responses within endothelial cells. Activation of NF-κB, MAPKs, and downstream cytokine production promotes endothelial activation, while RhoA/ROCK-mediated cytoskeletal contraction induces intercellular gap formation and barrier disruption *[36]*. These mechanisms are likely to cause the reduction in TER observed following LPS exposure in our study. Furthermore, inflammatory signaling initiated by LPS is known to promote glycocalyx shedding [*45,46*], contractile responses and destabilization of endothelial junctions, thereby amplifying vascular permeability [*47,48*].

PLY, a pore-forming, cholesterol-dependent cytotoxin produced by Streptococcus pneumoniae, represents another major mediator of pulmonary endothelial dysfunction and a key driver of permeability edema during pneumococcal pneumonia [*49*]. Consistently, PLY treatment in our study resulted in disruption of the EC barrier function *In-vitro* and vascular leak in the lungs of mice.

Mechanistically, PLY forms transmembrane pores that cause rapid calcium influx and disruption of intracellular ion homeostasis [*50,51*]. Elevated intracellular calcium activates signaling pathways that promote actomyosin contraction, stress fiber formation, and adherens junction disassembly *[18] [29]*. In particular, PLY induces endothelial hyperpermeability through activation of PKC-α, arginase-1, and RhoA-dependent signaling pathways [*18*]. Recent studies have further expanded this model by demonstrating that PLY-induced endothelial injury is amplified by generation of reactive oxygen species (ROS) as well as redox-sensitive signaling pathways. It was shown that Nox1-derived ROS plays a role in PLY-mediated barrier dysfunction, suggesting that oxidative stress acts synergistically with calcium signaling to promote vascular leak [*19*]. Collectively, these findings suggest that endothelial injury caused by PLY results from multiple interacting mechanisms, including pore formation, oxidative stress, inflammatory signaling, and cytoskeletal remodeling.

An important aspect of PLY- and LPS-induced injury involves degradation of the endothelial glycocalyx. This structure functions as the first line of defense against circulating inflammatory mediators and mechanical stress *[12] [52]*. Compromised glycocalyx integrity increases the susceptibility of endothelial cells to inflammatory activation and permeability responses *[53] [54] [55]*. Recent reviews of pulmonary endothelial dysfunction have highlighted glycocalyx degradation as a central mechanism contributing to vascular leak in ALI and ARDS *[56] [57] [58]*. Therefore, preservation of glycocalyx structure has emerged as an attractive therapeutic target in the management of inflammatory lung diseases *[59] [60]*.

This study demonstrates that sulodexide, efficiently protects endothelial cells against both LPS- and PLY-induced injury. SDX is a glycosaminoglycan compound made up mainly of low-molecular-weight heparin and dermatan sulfate [*61]* and is widely recognized for its endothelial-protective, anti-inflammatory, and glycocalyx-restorative properties *[21,20]*. Our findings suggest that maintenance of glycocalyx integrity plays a crucial role in limiting endothelial responses to bacterial toxins and consistent with previous studies demonstrating that preservation of the endothelial glycocalyx decreases vascular permeability *[62] [63] [59,54]*.

Several mechanisms may be responsible for the protective effects of SDX. Previous studies have shown that SDX inhibits glycocalyx shedding by suppressing heparanase activity, matrix metalloproteinases, and oxidative stress pathways. Preservation of the glycocalyx likely reduces direct exposure of endothelial receptors to inflammatory mediators and restricts activation of permeability-inducing signaling cascades. Furthermore, glycocalyx preservation may improve mechanotransduction and stabilize membrane microdomains involved in endothelial barrier regulation *[16]*.

In addition to its glycocalyx-preserving properties, SDX has been shown to directly modulate inflammatory signaling pathways associated with endothelial barrier dysfunction. Giurdanella et al. *[24]* demonstrated that SDX protected human retinal endothelial cells from injury induced by high glucose and AGEs, by suppressing activation of the ERK/cPLA$_2$/COX-2/PGE$_2$ inflammatory pathway and suppressing NF-κB signaling. SDX preserved endothelial junctional proteins, including VE-cadherin, occludin, and claudin-5, improved transendothelial electrical resistance, and reduced endothelial permeability. Importantly, these protective effects were attributed to the suppression of AGE/RAGE-mediated signaling, suggesting that SDX not only restores glycocalyx integrity but also mitigates downstream inflammatory pathways that promote endothelial barrier disruption *[56] [20,21]*. SDX not only prevents inflammatory injury but may also strengthen endothelial barrier properties *[20] [23] [16]*. Improved glycocalyx integrity, enhanced junctional stability, and reduced basal inflammatory signaling may collectively contribute to this effect. Such barrier-enhancing properties may be particularly valuable in clinical trials where endothelial dysfunction predisposes patients to severe inflammatory responses *[52,12]*. These findings are highly relevant to the present study and support the concept that SDX exerts broad endothelial-protective effects across different vascular beds by limiting inflammatory activation and preserving intercellular junctional integrity.

In our ECIS experiments, SDX significantly attenuates the decrease in TER induced by LPS and PLY. Further, SDX post-treatment restores TER after PLY insult. It consistent with the data from literature indicating that preservation of glycocalyx integrity directly contributes to maintenance of the endothelial barrier *[54] [64]*. Our immunofluorescence findings further support this protective mechanism. Exposure to LPS or PLY causes marked disruption of VE-cadherin localization at cell-cell junctions and induces F=actin stress fiber formation. As a key component of endothelial adherens junctions, VE-cadherin disruption is closely associated with elevated vascular permeability *[65,66]*. Simultaneously, formation of actin stress fibers reflects activation of contractile pathways that generate tension within endothelial cells and promote intercellular gap formation. In contrast, SDX-treated cells exhibited more continuous VE-cadherin staining and reduced F-actin stress fiber formation, indicating preservation of junctional stability and cytoskeletal organization *[33]*. These structural observations correlate closely with the TER experiments and suggest that SDX maintains endothelial barrier integrity at both functional and molecular levels and also found similar findings where SDX attenuates the effect of LPS and PLY induced ALI *[67] [21] [20] [23]*.

Notably, that SDX is less efficient against LPS as compared to PLY. The reason for this is not clear, but may be explained, at least in part, by the fact that SDX may directly (or via strengthening glycocalyx)

affect membrane pore formation induced by PLY, thereby inhibiting downstream signaling events. In contrast, to PLY, LPS signaling entirely depends upon receptor activation, therefore, is less susceptible to SDX-mediated glycocalyx strengthening. However, this hypothesis requires further investigation. Another possibility is that SDX directly neutralized PLY. Novel PLY inhibitors capable of neutralizing toxin activity have shown promising in reducing cellular injury and preserving endothelial function*[68] [69]*. However, it is unlikely in the case of SDX, since it reserves the effect of PLY on EC permeability.

In-vitro findings on EC barrier protective effects of SDX were supported by the *In-vivo* Evans Blue Dye permeability studies, where SDX significantly reduced LPS- and PLY-induced vascular leakage into lung tissue, demonstrating its ability to preserve pulmonary vascular integrity under inflammatory conditions. Interestingly, despite the reduction in vascular permeability, SDX treatment does not significantly alter LPS-induced incrreas4e in total protein concentration within bronchoalveolar lavage fluid. This observation suggests that while SDX efficiently protects the endothelial layer thus limiting vascular leak, BALF protein accumulation may additionally reflect epithelial barrier injury, inflammatory exudation, and impaired alveolar fluid clearance, processes that may not be fully corrected by intravenous SDX instillation. It well established that intratracheal LPS caused epithelial damage *[70]*.

Taken together, these findings suggest that maintaining glycocalyx integrity represents a crucial mechanism by which SDX limits endothelial responses to bacterial toxins and preserves pulmonary vascular barrier function.

The clinical implications of these findings are significant. Endothelial dysfunction and glycocalyx degradation are increasingly recognized as major contributors to sepsis, bacterial pneumonia, ALI, and ARDS *[12] [64]*. Current treatments for these diseases are largely supportive and do not directly address endothelial barrier failure. The ability of SDX to preserve glycocalyx integrity, maintain VE-cadherin-dependent junctions, reduce cytoskeletal remodeling, and improve endothelial barrier function suggests considerable therapeutic potential *[20] [21]*.

Conclusion

In conclusion, the present study demonstrates that bacterial toxins, LPS and PLY, induce profound pulmonary endothelial barrier dysfunction through mechanisms involving VE-cadherin downregulation, cytoskeletal remodeling, and increased endothelial permeability *In-vitro* and pulmonary vascular leak in mice. Pretreatment with SDX efficiently attenuates these detrimental effects likely by preserving glycocalyx integrity resulting in maintaining adherens junction organization, reducing stress fiber formation, and improving endothelial barrier function. Notably, that protective effects of SDX are more prominent against pore-forming toxins (PLY) as compared to receptor-mediated counterparts (LPS). Overall, our results support the concept that glycocalyx preservation represents a promising therapeutic strategy for preventing vascular leak and mitigating lung injury associated with bacterial pneumonia, ALI, and ARDS.

Statistical Analysis

Values are expressed as mean +/- SEM of at least 3-5 independent experiments and statistical analyses were performed using GraphPad Software (San Diego, CA). Analysis of variance (ANOVA) or Student's t test was used, as appropriate. A *P* value of < 0.05 and <0.0001 was considered statistically significant.

Abbreviations

ALI
Acute lung injury
EBD
Evans Blue Dye
ARDS
Acute Respiratory Distress Syndrome
HLMVECs
Human lung microvascular endothelial cells
SDX
Sulodexide
LPS
Lipopolysaccharide
PLY
Pneumolysin
TER
Transendothelial electrical resistance
VE-cadherin
Vascular endothelium cadherin

Declarations

Data availability statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgements

We thank ALFA WASSERMANN S.p.A., Bologna, Italy and Dr. Trinad Chakraborty (Institute for Medical Microbiology, Justus-Liebig University, Giessen, Germany, retired) for providing SDX and PLY, respectively, for current study, and Dr. Boris Gorshkov (Augusta University, retired) for providing technical assistance.

Funding

The sources of funding for the experiments are from RO1 grants (1R01HL157440-01A1; 1R01HL158909-01A1) and from Intramural funds (IGPP00077) for ADV, P01 grant (HL160557) AHA award

(23TPA1072536) and Intramural funds (IGPP00083) for RL, as well as AHA fellowships 26POST1557492 for LJ and 26POST1561113 for RSP. K99/R00 funding from the National Heart, Lung, and Blood Institute (1K99HL186240-01) for RSP.

Author's information

Authors and affiliations

Vascular Biology Center, Augusta University, Augusta, GA 30912, USA.

Latika Jaiswal, Peter Biggs, Mary Cherian-Shaw, Liselle Simon, Shweta R. Patil, Basmah Mohamed, Rahul Shivaji Patil, Anita Kovacs-Kasa, Rudolf Lucas, Alexander D Verin

Graduate School, Augusta University, Augusta, GA 30912, USA.

Peter Biggs

Department of Pharmacology and Toxicology, Augusta University, Augusta, GA 30912, USA

Rudolf Lucas

Department of Medicine, Augusta University, Augusta, GA 30912, USA

Anita Kovacs-Kasa, Alexander D Verin

Authors' contributions

ADV, AKK, RL: Conceptualization and Supervision. LJ, PB, MCS, AKK, BM, RSP, SRP, LS, RL, ADV: Methodology, Data Curation, Formal Analysis and Visualization. LJ, RSP, RL, ADV: Resources. LJ, ADV, RSP, RL: Project administration and Funding acquisition. LJ, PB: Drafting the manuscript. LJ, PB, MCS, AKK, BM, LS, RL, RSP, SRP, ADV: Edited and reviewed the manuscript.

Corresponding Author

Corresponding to Alexander D Verin (averin@augusta.edu).

Ethics Declaration

Ethical Approval

All procedures adhered to NIH guidelines and received prior approval from the Augusta University Institutional Animal Care and Use Committee.

Conflict of Interest

The authors declare no competing interest.

References

1. Johnson ER, Matthay MA (2010) Acute lung injury: epidemiology, pathogenesis, and treatment. J Aerosol Med Pulm Drug Deliv 23 (4):243–252. doi:10.1089/jamp.2009.0775
2. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ, Hudson LD (2005) Incidence and outcomes of acute lung injury. N Engl J Med 353 (16):1685–1693. doi:10.1056/NEJMoa050333
3. Butt Y, Kurdowska A, Allen TC (2016) Acute Lung Injury: A Clinical and Molecular Review. Arch Pathol Lab Med 140 (4):345–350. doi:10.5858/arpa.2015-0519-RA
4. Zhang W, Jiang L, Tong X, He H, Zheng Y, Xia Z (2024) Sepsis-Induced Endothelial Dysfunction: Permeability and Regulated Cell Death. J Inflamm Res 17:9953–9973. doi:10.2147/JIR.S479926
5. Mu S, Liu Y, Jiang J, Ding R, Li X, Li X, Ma X (2018) Unfractionated heparin ameliorates pulmonary microvascular endothelial barrier dysfunction via microtubule stabilization in acute lung injury. Respir Res 19 (1):220. doi:10.1186/s12931-018-0925-6
6. Hough RF, Alvira CM, Bastarache JA, Erzurum SC, Kuebler WM, Schmidt EP, Shimoda LA, Abman SH, Alvarez DF, Belvitch P, Bhattacharya J, Birukov KG, Chan SY, Cornfield DN, Dudek SM, Garcia JGN, Harrington EO, Hsia CCW, Islam MN, Jonigk DD, Kalinichenko VV, Kolb TM, Lee JY, Mammoto A, Mehta D, Rounds S, Schupp JC, Shaver CM, Suresh K, Tambe DT, Ventetuolo CE, Yoder MC, Stevens T, Damarla M (2024) Studying the Pulmonary Endothelium in Health and Disease. An Official American Thoracic Society Workshop Report. Am J Respir Cell Mol Biol. doi:10.1165/rcmb.2024-0330ST
7. Chelazzi C, Villa G, Mancinelli P, De Gaudio AR, Adembri C (2015) Glycocalyx and sepsis-induced alterations in vascular permeability. Crit Care 19 (1):26. doi:10.1186/s13054-015-0741-z
8. Lubkin A, Torres VJ (2017) Bacteria and endothelial cells: a toxic relationship. Curr Opin Microbiol 35:58–63. doi:10.1016/j.mib.2016.11.008
9. Goligorsky MS, Sun D (2020) Glycocalyx in Endotoxemia and Sepsis. Am J Pathol 190 (4):791–798. doi:10.1016/j.ajpath.2019.06.017
10. Curry FE, Adamson RH (2012) Endothelial glycocalyx: permeability barrier and mechanosensor. Ann Biomed Eng 40 (4):828–839. doi:10.1007/s10439-011-0429-8
11. Claesson-Welsh L, Dejana E, McDonald DM (2021) Permeability of the Endothelial Barrier: Identifying and Reconciling Controversies. Trends Mol Med 27 (4):314–331. doi:10.1016/j.molmed.2020.11.006
12. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG (2007) The endothelial glycocalyx: composition, functions, and visualization. Pflugers Arch 454 (3):345–359. doi:10.1007/s00424-007-0212-8
13. Kim YH, Nijst P, Kiefer K, Tang WH (2017) Endothelial Glycocalyx as Biomarker for Cardiovascular Diseases: Mechanistic and Clinical Implications. Curr Heart Fail Rep 14 (2):117–126. doi:10.1007/s11897-017-0320-5

14. Ying J, Zhang C, Wang Y, Liu T, Yu Z, Wang K, Chen W, Zhou Y, Lu G (2023) Sulodexide improves vascular permeability via glycocalyx remodelling in endothelial cells during sepsis. Front Immunol 14:1172892. doi:10.3389/fimmu.2023.1172892
15. Sallee CJ, Maddux AB, Hippensteel JA, Markovic D, Oshima K, Schwingshackl A, Mourani PM, Schmidt EP, Sapru A (2024) Circulating Heparan Sulfate Profiles in Pediatric Acute Respiratory Distress Syndrome. Shock 62 (4):496–504. doi:10.1097/SHK.0000000000002421
16. Song JW, Zullo JA, Liveris D, Dragovich M, Zhang XF, Goligorsky MS (2017) Therapeutic Restoration of Endothelial Glycocalyx in Sepsis. J Pharmacol Exp Ther 361 (1):115–121. doi:10.1124/jpet.116.239509
17. Tauseef M, Knezevic N, Chava KR, Smith M, Sukriti S, Gianaris N, Obukhov AG, Vogel SM, Schraufnagel DE, Dietrich A, Birnbaumer L, Malik AB, Mehta D (2012) TLR4 activation of TRPC6-dependent calcium signaling mediates endotoxin-induced lung vascular permeability and inflammation. J Exp Med 209 (11):1953–1968. doi:10.1084/jem.20111355
18. Lucas R, Yang G, Gorshkov BA, Zemskov EA, Sridhar S, Umapathy NS, Jezierska-Drutel A, Alieva IB, Leustik M, Hossain H, Fischer B, Catravas JD, Verin AD, Pittet JF, Caldwell RB, Mitchell TJ, Cederbaum SD, Fulton DJ, Matthay MA, Caldwell RW, Romero MJ, Chakraborty T (2012) Protein kinase C-alpha and arginase I mediate pneumolysin-induced pulmonary endothelial hyperpermeability. Am J Respir Cell Mol Biol 47 (4):445–453. doi:10.1165/rcmb.2011-0332OC
19. Czikora I, Alli AA, Sridhar S, Matthay MA, Pillich H, Hudel M, Berisha B, Gorshkov B, Romero MJ, Gonzales J, Wu G, Huo Y, Su Y, Verin AD, Fulton D, Chakraborty T, Eaton DC, Lucas R (2017) Epithelial Sodium Channel-alpha Mediates the Protective Effect of the TNF-Derived TIP Peptide in Pneumolysin-Induced Endothelial Barrier Dysfunction. Front Immunol 8:842. doi:10.3389/fimmu.2017.00842
20. Broekhuizen LN, Lemkes BA, Mooij HL, Meuwese MC, Verberne H, Holleman F, Schlingemann RO, Nieuwdorp M, Stroes ES, Vink H (2010) Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 diabetes mellitus. Diabetologia 53 (12):2646–2655. doi:10.1007/s00125-010-1910-x
21. Coccheri S, Mannello F (2013) Development and use of sulodexide in vascular diseases: implications for treatment. Drug Des Devel Ther 8:49–65. doi:10.2147/DDDT.S6762
22. Ciszewicz M, Polubinska A, Antoniewicz A, Suminska-Jasinska K, Breborowicz A (2009) Sulodexide suppresses inflammation in human endothelial cells and prevents glucose cytotoxicity. Transl Res 153 (3):118–123. doi:10.1016/j.trsl.2008.12.007
23. Mannello F, Ligi D, Canale M, Raffetto JD (2014) Sulodexide down-regulates the release of cytokines, chemokines, and leukocyte colony stimulating factors from human macrophages: role of glycosaminoglycans in inflammatory pathways of chronic venous disease. Curr Vasc Pharmacol 12 (1):173–185. doi:10.2174/15701611116661311261440 25
24. Giurdanella G, Lazzara F, Caporarello N, Lupo G, Anfuso CD, Eandi CM, Leggio GM, Drago F, Bucolo C, Salomone S (2017) Sulodexide prevents activation of the PLA2/COX-2/VEGF inflammatory pathway

in human retinal endothelial cells by blocking the effect of AGE/RAGE. Biochem Pharmacol 142:145–154. doi:10.1016/j.bcp.2017.06.130

25. Tiruppathi C, Malik AB, Del Vecchio PJ, Keese CR, Giaever I (1992) Electrical method for detection of endothelial cell shape change in real time: assessment of endothelial barrier function. Proceedings of the National Academy of Sciences of the United States of America 89 (17):7919–7923
26. Moitra J, Sammani S, Garcia JG (2007) Re-evaluation of Evans Blue dye as a marker of albumin clearance in murine models of acute lung injury. Transl Res 150 (4):253–265. doi:10.1016/j.trsl.2007.03.013
27. Lucas R, Verin AD, Black SM, Catravas JD (2009) Regulators of endothelial and epithelial barrier integrity and function in acute lung injury. Biochem Pharmacol 77 (12):1763–1772. doi:10.1016/j.bcp.2009.01.014
28. Batori RK, Chen F, Bordan Z, Haigh S, Su Y, Verin AD, Barman SA, Stepp DW, Chakraborty T, Lucas R, Fulton DJR (2022) Protective role of Cav-1 in pneumolysin-induced endothelial barrier dysfunction. Front Immunol 13:945656. doi:10.3389/fimmu.2022.945656
29. Witzenrath M, Gutbier B, Hocke AC, Schmeck B, Hippenstiel S, Berger K, Mitchell TJ, de los Toyos JR, Rosseau S, Suttorp N, Schutte H (2006) Role of pneumolysin for the development of acute lung injury in pneumococcal pneumonia. Crit Care Med 34 (7):1947–1954. doi:10.1097/01.CCM.0000220496.48295.A9
30. Kovacs-Kasa A, Gorshkov BA, Kim KM, Kumar S, Black SM, Fulton DJ, Dimitropoulou C, Catravas JD, Verin AD (2016) The protective role of MLCP-mediated ERM dephosphorylation in endotoxin-induced lung injury in vitro and in vivo. Sci Rep 6:39018. doi:10.1038/srep39018
31. Bogatcheva NV, Zemskova MA, Poirier C, Mirzapoiazova T, Kolosova I, Bresnick AR, Verin AD (2011) The suppression of myosin light chain (MLC) phosphorylation during the response to lipopolysaccharide (LPS): beneficial or detrimental to endothelial barrier? J Cell Physiol 226 (12):3132–3146. doi:10.1002/jcp.22669
32. Bogatcheva NV, Zemskova MA, Kovalenkov Y, Poirier C, Verin AD (2009) Molecular mechanisms mediating protective effect of cAMP on lipopolysaccharide (LPS)-induced human lung microvascular endothelial cells (HLMVEC) hyperpermeability. J Cell Physiol 221 (3):750–759. doi:10.1002/jcp.21913
33. Komarova Y, Malik AB (2010) Regulation of endothelial permeability via paracellular and transcellular transport pathways. Annu Rev Physiol 72:463–493. doi:10.1146/annurev-physiol-021909-135833
34. Lucas R, Sridhar S, Rick FG, Gorshkov B, Umapathy NS, Yang G, Oseghale A, Verin AD, Chakraborty T, Matthay MA, Zemskov EA, White R, Block NL, Schally AV (2012) Agonist of growth hormone-releasing hormone reduces pneumolysin-induced pulmonary permeability edema. Proc Natl Acad Sci U S A 109 (6):2084–2089. doi:10.1073/pnas.1121075109
35. Birukova AA, Tian X, Cokic I, Beckham Y, Gardel ML, Birukov KG (2013) Endothelial barrier disruption and recovery is controlled by substrate stiffness. Microvasc Res 87:50–57.

doi:10.1016/j.mvr.2012.12.006

36. Wan J, Shan Y, Fan Y, Fan C, Chen S, Sun J, Zhu L, Qin L, Yu M, Lin Z (2016) NF-kappaB inhibition attenuates LPS-induced TLR4 activation in monocyte cells. Mol Med Rep 14 (5):4505–4510. doi:10.3892/mmr.2016.5825
37. Gonzales JN, Gorshkov B, Varn MN, Zemskova MA, Zemskov EA, Sridhar S, Lucas R, Verin AD (2014) Protective effect of adenosine receptors against lipopolysaccharide-induced acute lung injury. Am J Physiol Lung Cell Mol Physiol 306 (6):L497–507. doi:10.1152/ajplung.00086.2013
38. Kovacs-Kasa A, Kovacs L, Cherian-Shaw M, Patel V, Meadows ML, Fulton DJ, Su Y, Verin AD (2021) Inhibition of Class IIa HDACs improves endothelial barrier function in endotoxin-induced acute lung injury. J Cell Physiol 236 (4):2893–2905. doi:10.1002/jcp.30053
39. Czikora I, Sridhar S, Gorshkov B, Alieva IB, Kasa A, Gonzales J, Potapenko O, Umapathy NS, Pillich H, Rick FG, Block NL, Verin AD, Chakraborty T, Matthay MA, Schally AV, Lucas R (2014) Protective effect of Growth Hormone-Releasing Hormone agonist in bacterial toxin-induced pulmonary barrier dysfunction. Front Physiol 5:259. doi:10.3389/fphys.2014.00259
40. Romero MJ, Yue Q, Ahn WM, Hamacher J, Zaidi Y, Haigh S, Sridhar S, Gonzales J, Hudel M, Huo Y, Verin AD, Pace BS, Stansfield BK, Maishan M, Neptune ER, Enkhbaatar P, Su Y, Chakraborty T, Gonsalvez G, Hummler E, Davis WB, Bogdanov VY, Fulton DJR, Csanyi G, Matthay MA, Eaton DC, Lucas R (2025) Endothelial ENaC-alpha Restrains Oxidative Stress in Lung Capillaries in Murine Pneumococcal Pneumonia-associated Acute Lung Injury. Am J Respir Cell Mol Biol 72 (4):429–440. doi:10.1165/rcmb.2023-0440OC
41. Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, Kuebler WM, Acute Lung Injury in Animals Study G (2011) An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. Am J Respir Cell Mol Biol 44 (5):725–738. doi:10.1165/rcmb.2009-0210ST
42. Eutamene H, Theodorou V, Schmidlin F, Tondereau V, Garcia-Villar R, Salvador-Cartier C, Chovet M, Bertrand C, Bueno L (2005) LPS-induced lung inflammation is linked to increased epithelial permeability: role of MLCK. Eur Respir J 25 (5):789–796. doi:10.1183/09031936.05.00064704
43. Li XY, Donaldson K, MacNee W (1998) Lipopolysaccharide-induced alveolar epithelial permeability: the role of nitric oxide. Am J Respir Crit Care Med 157 (4 Pt 1):1027–1033. doi:10.1164/ajrccm.157.4.9605080
44. Opal SM, van der Poll T (2015) Endothelial barrier dysfunction in septic shock. J Intern Med 277 (3):277–293. doi:10.1111/joim.12331
45. Huang L, Zhang X, Ma X, Zhang D, Li D, Feng J, Pan X, Lu J, Wang X, Liu X (2018) Berberine alleviates endothelial glycocalyx degradation and promotes glycocalyx restoration in LPS-induced ARDS. Int Immunopharmacol 65:96–107. doi:10.1016/j.intimp.2018.10.001
46. Taghavi S, Abdullah S, Duchesne J, Pociask D, Kolls J, Jackson-Weaver O (2021) Interleukin 22 mitigates endothelial glycocalyx shedding after lipopolysaccharide injury. J Trauma Acute Care Surg 90 (2):337–345. doi:10.1097/TA.0000000000003019

47. Wang M, Feng J, Zhou D, Wang J (2023) Bacterial lipopolysaccharide-induced endothelial activation and dysfunction: a new predictive and therapeutic paradigm for sepsis. Eur J Med Res 28 (1):339. doi:10.1186/s40001-023-01301-5
48. Kasa A, Csortos C, Verin AD (2015) Cytoskeletal mechanisms regulating vascular endothelial barrier function in response to acute lung injury. Tissue Barriers 3 (1-2):e974448. doi:10.4161/21688370.2014.974448
49. Lucas R, Czikora I, Sridhar S, Zemskov E, Gorshkov B, Siddaramappa U, Oseghale A, Lawson J, Verin A, Rick FG, Block NL, Pillich H, Romero M, Leustik M, Schally AV, Chakraborty T (2013) Mini-review: novel therapeutic strategies to blunt actions of pneumolysin in the lungs. Toxins (Basel) 5 (7):1244–1260. doi:10.3390/toxins5071244
50. Pereira JM, Xu S, Leong JM, Sousa S (2022) The Yin and Yang of Pneumolysin During Pneumococcal Infection. Front Immunol 13:878244. doi:10.3389/fimmu.2022.878244
51. Nishimoto AT, Rosch JW, Tuomanen EI (2020) Pneumolysin: Pathogenesis and Therapeutic Target. Front Microbiol 11:1543. doi:10.3389/fmicb.2020.01543
52. Weinbaum S, Tarbell JM, Damiano ER (2007) The structure and function of the endothelial glycocalyx layer. Annu Rev Biomed Eng 9:121–167. doi:10.1146/annurev.bioeng.9.060906.151959
53. Becker BF, Chappell D, Jacob M (2010) Endothelial glycocalyx and coronary vascular permeability: the fringe benefit. Basic Res Cardiol 105 (6):687–701. doi:10.1007/s00395-010-0118-z
54. Uchimido R, Schmidt EP, Shapiro NI (2019) The glycocalyx: a novel diagnostic and therapeutic target in sepsis. Crit Care 23 (1):16. doi:10.1186/s13054-018-2292-6
55. Anderson R, Nel JG, Feldman C (2018) Multifaceted Role of Pneumolysin in the Pathogenesis of Myocardial Injury in Community-Acquired Pneumonia. Int J Mol Sci 19 (4). doi:10.3390/ijms19041147
56. Li H, Wen H, Liu J, Luo X, Pei B, Ge P, Sun Z, Liu J, Wang J, Chen H (2025) The glycocalyx: a key target for treatment of severe acute pancreatitis-associated multiple organ dysfunction syndrome. Hum Cell 38 (4):107. doi:10.1007/s13577-025-01227-6
57. Babu MA, Rekha A, Goyal K, Menon SV, Ray S, Uniyal P, Maji C, Khan A (2026) Endothelial Glycocalyx biomarkers in acute lung injury. Clin Chim Acta 582:120804. doi:10.1016/j.cca.2025.120804
58. Qiao X, Yin J, Zheng Z, Li L, Feng X (2024) Endothelial cell dynamics in sepsis-induced acute lung injury and acute respiratory distress syndrome: pathogenesis and therapeutic implications. Cell Commun Signal 22 (1):241. doi:10.1186/s12964-024-01620-y
59. Becker BF, Jacob M, Leipert S, Salmon AH, Chappell D (2015) Degradation of the endothelial glycocalyx in clinical settings: searching for the sheddases. Br J Clin Pharmacol 80 (3):389–402. doi:10.1111/bcp.12629
60. Martin L, Koczera P, Zechendorf E, Schuerholz T (2016) The Endothelial Glycocalyx: New Diagnostic and Therapeutic Approaches in Sepsis. Biomed Res Int 2016:3758278. doi:10.1155/2016/3758278
61. Lauver DA, Lucchesi BR (2006) Sulodexide: a renewed interest in this glycosaminoglycan. Cardiovasc Drug Rev 24 (3-4):214–226. doi:10.1111/j.1527-3466.2006.00214.x

62. Collins SR, Blank RS, Deatherage LS, Dull RO (2013) Special article: the endothelial glycocalyx: emerging concepts in pulmonary edema and acute lung injury. Anesth Analg 117 (3):664–674. doi:10.1213/ANE.0b013e3182975b85
63. Sieve I, Munster-Kuhnel AK, Hilfiker-Kleiner D (2018) Regulation and function of endothelial glycocalyx layer in vascular diseases. Vascul Pharmacol 100:26–33. doi:10.1016/j.vph.2017.09.002
64. Schmidt EP, Yang Y, Janssen WJ, Gandjeva A, Perez MJ, Barthel L, Zemans RL, Bowman JC, Koyanagi DE, Yunt ZX, Smith LP, Cheng SS, Overdier KH, Thompson KR, Geraci MW, Douglas IS, Pearse DB, Tuder RM (2012) The pulmonary endothelial glycocalyx regulates neutrophil adhesion and lung injury during experimental sepsis. Nat Med 18 (8):1217–1223. doi:10.1038/nm.2843
65. Dejana E, Orsenigo F, Lampugnani MG (2008) The role of adherens junctions and VE-cadherin in the control of vascular permeability. J Cell Sci 121 (Pt 13):2115–2122. doi:10.1242/jcs.017897
66. Vestweber D (2008) VE-cadherin: the major endothelial adhesion molecule controlling cellular junctions and blood vessel formation. Arterioscler Thromb Vasc Biol 28 (2):223–232. doi:10.1161/ATVBAHA.107.158014
67. Xie L, Song M, Deng Z, He Q, Chen S, Zhang T, Kuang Y, Jing X, Liu S, Fang C, Zhang B (2026) Sulodexide attenuates sepsis-induced lung injury in neonatal rats via TNF-alpha pathway. Pediatr Res. doi:10.1038/s41390-026-05067-4
68. Cima Cabal MD, Molina F, Lopez-Sanchez JI, Perez-Santin E, Del Mar Garcia-Suarez M (2023) Pneumolysin as a target for new therapies against pneumococcal infections: A systematic review. PLoS One 18 (3):e0282970. doi:10.1371/journal.pone.0282970
69. Witzenrath M, Gutbier B, Schmeck B, Tenor H, Seybold J, Kuelzer R, Grentzmann G, Hatzelmann A, van Laak V, Tschernig T, Mitchell TJ, Schudt C, Rosseau S, Suttorp N, Schutte H (2009) Phosphodiesterase 2 inhibition diminished acute lung injury in murine pneumococcal pneumonia. Crit Care Med 37 (2):584–590. doi:10.1097/CCM.0b013e3181959814
70. Menezes SL, Bozza PT, Neto HC, Laranjeira AP, Negri EM, Capelozzi VL, Zin WA, Rocco PR (2005) Pulmonary and extrapulmonary acute lung injury: inflammatory and ultrastructural analyses. J Appl Physiol (1985) 98 (5):1777–1783. doi:10.1152/japplphysiol.01182.2004

Figures

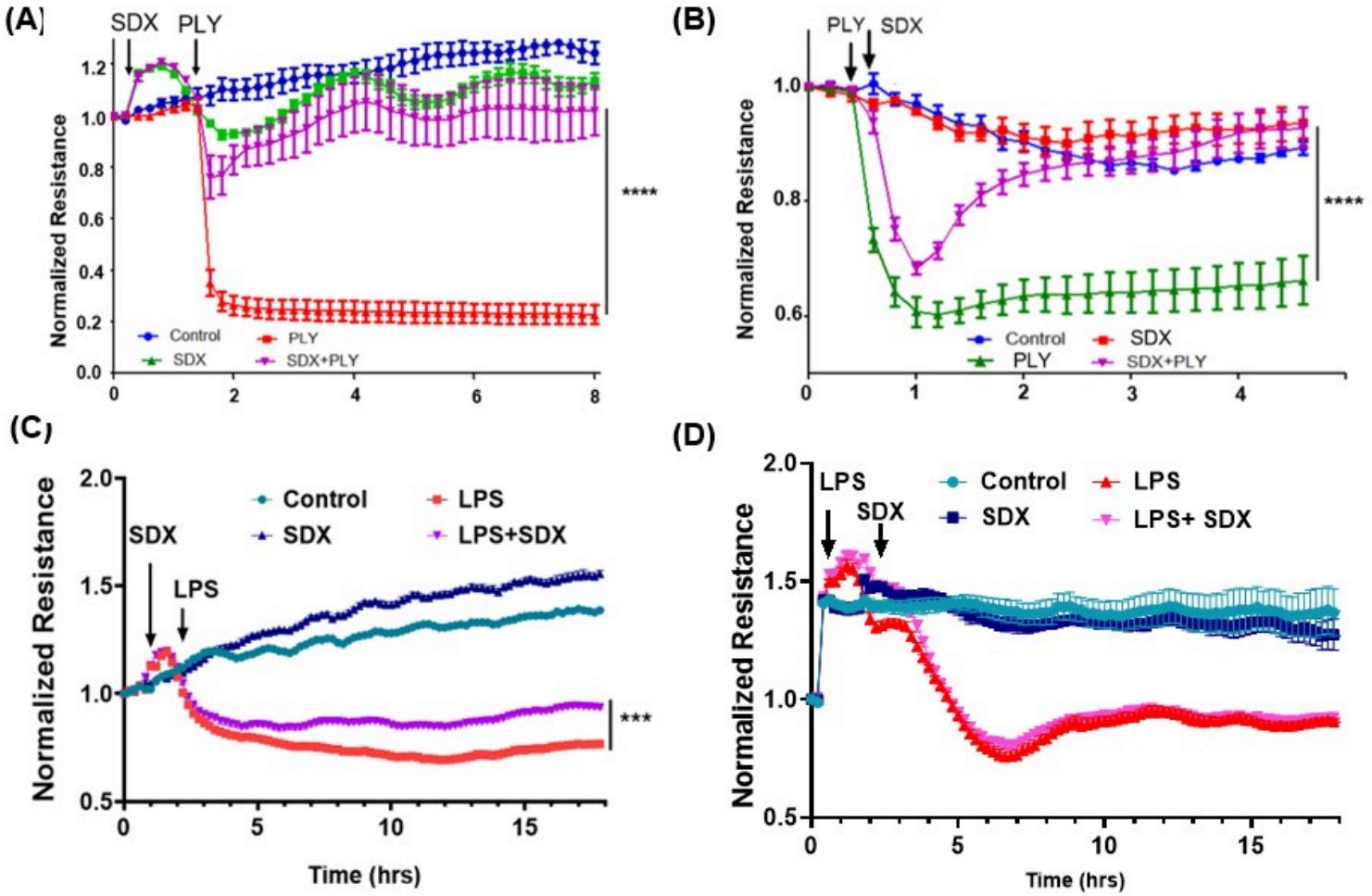


Figure 1

The effect of SDX on HLMVEC permeability induced by bacterial toxins, PLY and LPS. Real-time transendothelial electrical resistance (TER) measurements in HLMVEC monolayers treated with vehicle (PBS), PLY (60 ng/ml), LPS (100 e.u.) in serum-free media, +/- SDX (150 µg/ml), or SDX alone. (A)TER in HLMVECs pretreated with SDX for 1 hour prior to PLY. Arrows indicate time of SDX and PLY addition. (B) SDX administered 6 minutes after PLY addition. Arrows indicate time of PLY and SDX addition. (C)TER in HLMVECs pretreated with SDX for 1hr prior to LPS. Arrows indicate time of each addition. (D) TER in HLMVECs treated with SDX 1 hour after LPS addition. Arrows indicate time of SDX and LPS addition; n=4; Mean ± SEM. ****P<0.0001 and ***P<0.0001 considered significant by two-way ANOVA with Tukey's multiple comparisons test.

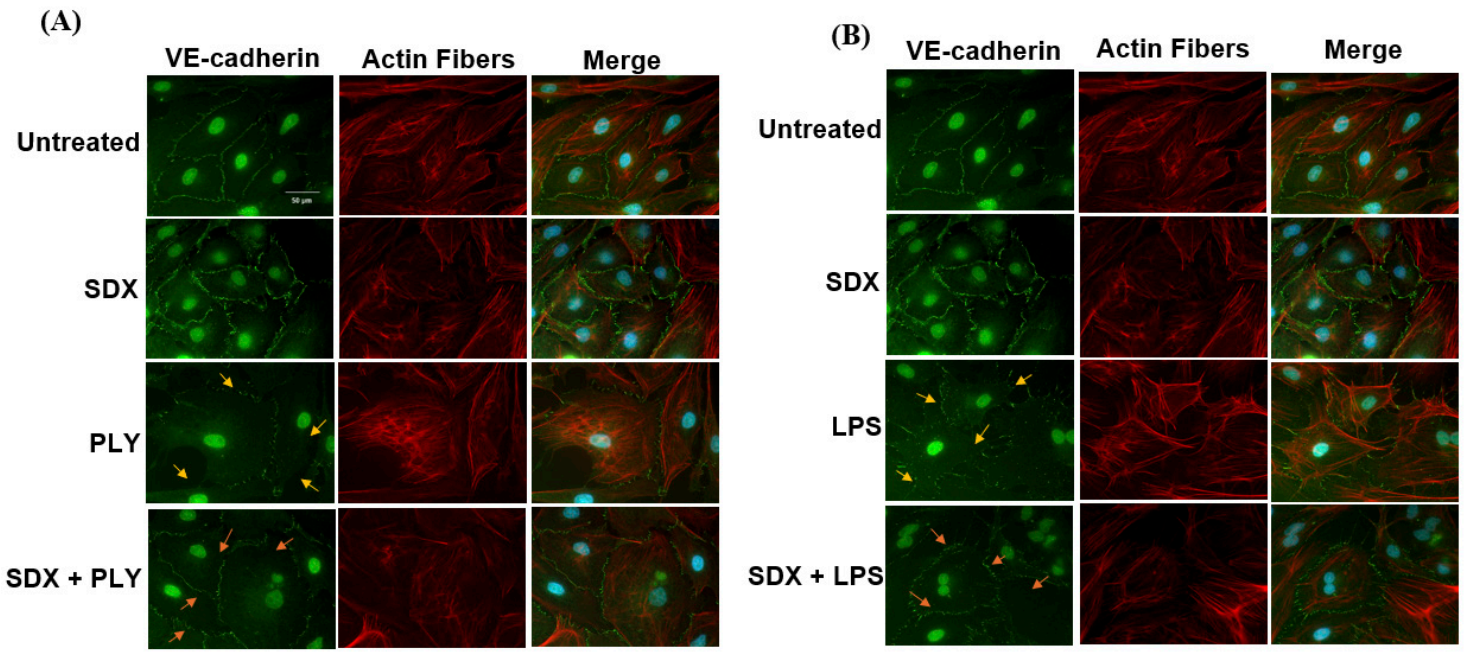


Figure 2

The effect of SDX on HLMVEC barrier integrity and cytoskeletal remodeling induced by PLY and LPS. (A) Representative (n=3) immunofluorescence images of HLMVECs stained for VE-cadherin (green), F-actin (red), and nuclei (DAPI; blue) following treatment with PLY (60 ng/ml) ± SDX (150 µg/ml). Cells were fixed in 4% PFA and stained with WGA (4 µg/ml) in Ca^{2+}/Mg^{2+}-supplemented HBSS for 30 minutes. Scale bar, 50 µm. (B) Representative (n=3) immunofluorescence images of HLMVECs stained for VE-cadherin (green), F-actin (red), and nuclei (DAPI; blue) following treatment with LPS (660 EU/ml, 6 hours) ± SDX (150 µg/ml). Yellow arrows indicates discontinuous, punctate VE-cadherin staining and gaps in junctional continuity at cell-cell borders, reflecting disruption of adherens junctions by LPS/PLY. Orange arrows indicates continuous, linear VE-cadherin staining along cell-cell borders at comparable positions, indicating preservation of junctional integrity in presence of SDX over LPS/PLY challenge.

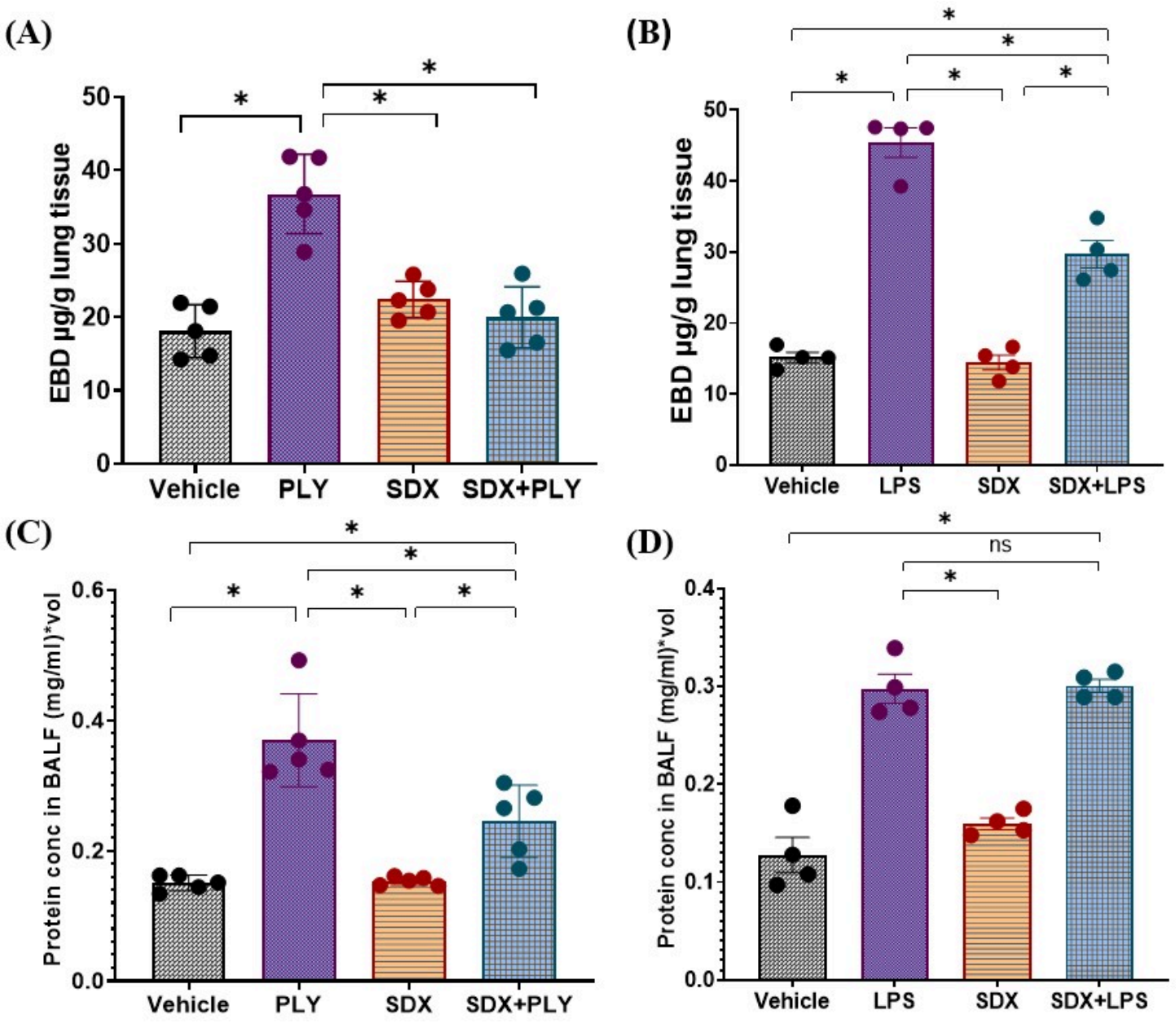


Figure 3

The effect of SDX on lung vascular dysfunction induced by PLY and LPS in mice. A, B: Bar graphs demonstrated EBD-albumin accumulation in the lung tissue. (A) Mice were subjected to intravenous administration of vehicle (PBS) or SDX (10 mg/kg body weight), 1hr prior to PLY (intratracheally, 2.7 µg/kg body weight) and were analyzed for EBD albumin. (B) Mice subjected to intravenous administration of vehicle (PBS) or SDX, (10 mg/kg body weight) 1hr prior to LPS (1mg/kg body weight) were analyzed for EBD-albumin accumulation in the lung tissue. Shown are mean ± SEM, n=4-5. *p<0.05 are considered significantly different. C, D: Bar graphs show protein levels in BALF (an index of lung inflammation and capillary leak). (C) Mice were injected with SDX (10 mg/kg body weight.) 1 hr through tail vein prior to PLY (2.7 µg/kg body weight.) i.t. (D) Mice were injected with SDX for 1hr through tail vein prior to LPS (1 mg/kg body weight.) i.t. Mean +/_ SEM; n=4; *P < 0.05. *P < 0.05, considered significant to control, significant to LPS and PLY.