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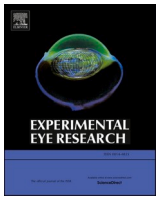

Research article

Liraglutide preserves endothelial function in ophthalmic arteries of septic mice via prevention of oxidative stress

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ABSTRACT

We hypothesized that the glucagon-like peptide-1 (GLP-1) receptor agonist liraglutide enhances endothelial functions in ophthalmic arteries of mice with polymicrobial sepsis. The study involved three groups of mice. Two groups underwent cecal ligation and puncture (CLP) to induce polymicrobial sepsis, with one of these groups receiving liraglutide treatment. The third group underwent sham surgery as a control. 24 h after the CLP procedure, the mice were euthanized, and ophthalmic arteries were isolated either for in vitro analysis of vascular function using videomicroscopy or for quantification of reactive oxygen species and redox genes or enzymes by PCR or immunohistochemistry. Vasoconstriction responses to the α_1-adrenoceptor agonist phenylephrine and vasodilation responses to sodium nitroprusside, an endothelium-independent vasodilator, were similar across all three groups. However, responses to acetylcholine, an endothelium-dependent vasodilator, were significantly impaired in ophthalmic arteries of septic mice. Notably, acetylcholine responses were preserved in septic mice treated with liraglutide. Additionally, dihydroethidium, and NOX2 antibody staining revealed a significant increase in markers for oxidative stress in the ophthalmic arteries of endotoxaemic mice, but not in those treated with liraglutide. In conclusion, activation of the GLP-1 receptor by liraglutide restrains endothelial dysfunction, reduces oxidative stress, and inhibits NOX2 overexpression in the ophthalmic arteries of endotoxaemic mice. These data suggest that GLP-1 receptor activation may provide a promising therapeutic strategy for treating ocular diseases characterized by oxidative stress and endothelial dysfunction.

1. Introduction

The peptide hormone analogue glucagon-like peptide-1 (GLP-1) plays a central function in the regulation of glucose metabolism. Released by intestinal enteroendocrine cells, GLP-1 stimulates its receptor on pancreatic β-cells, leading to the glucose-dependent secretion of insulin. Additionally, GLP-1 inhibits the release of glucagon from α-cells, which decelerates gastric emptying and supports the prevention of postprandial hyperglycemia (Helmstädter et al., 2022; Wettergren et al., 1993). Binding of GLP-1 to its receptor, a member of family B of G-protein-coupled receptors, activates the adenylate cyclase pathway, stimulating insulin synthesis and release (Drucker et al., 1987; Helmstädter et al., 2022). While predominantly expressed in pancreatic cells, expression of GLP-1 receptors was also detected in different tissues, including cells of the cardiovascular system (Helmstädter et al., 2022).

GLP-1 receptor analogs, such as liraglutide, exhibiting resistance to breakdown by the enzyme dipeptidyl peptidase-4 (DPP-4), have become a cornerstone in the evidence-based treatment of type 2 diabetes mellitus (T2DM). Multiple clinical trials have reported their safety and efficacy in managing blood glucose levels in patients with T2DM (Dungan et al., 2014; Galindo et al., 2023; Kirkman et al., 2024; Krishnan et al., 2024; Ma et al., 2024; Marso et al., 2016b). Furthermore, GLP-1 receptor agonists have been shown to significantly reduce the incidence of major

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adverse cardiovascular events in T2DM patients at high cardiovascular risk (Gerstein et al., 2019; Hernandez et al., 2018; Marso et al., 2016a, 2016b).

Sepsis, a life-threatening organ dysfunction initiated by an exaggerated response of the immune system to an infection, aligns with high mortality, being involved in approximately 20 % of global deaths (Levy et al., 2012; Rudd et al., 2020). A key component of sepsis pathophysiology is microvascular dysfunction, which leads to microthrombosis and ischemic damage to vital organs (Anas et al., 2010). Notably, rapid improvement in microcirculation was observed in septic shock survivors, but not in non-survivors, suggesting that vascular recovery is crucial for patient prognosis (Sakr et al., 2004). Survivors of sepsis are also at increased risk for long-term cardiovascular complications, as well as physical and cognitive impairments (Shankar-Hari and Rubenfeld, 2016). Inflammation and oxidative stress, contributing to endothelial dysfunction, are considered as pivotal contributors in sepsis-related microvascular injury (Ávila-Gómez et al., 2024; Girardis et al., 2024; Helmstädter et al., 2021; Huang et al., 2024; Steven et al., 2015).

Microvascular complications in the ocular vascular bed can significantly impair vision in septic patients. Occlusion of retinal vessels or posterior ciliary arteries, supplying important ocular structures, as the choroid and optic nerve head, has been documented in this patient group (Acharya et al., 2020; Aldrees and Micieli, 2020; Damani et al., 2023; Raj et al., 2021; Vosoughi and Micieli, 2023). Previous studies from our research team in a porcine model with induction of acute respiratory distress syndrome (ARDS) by intratracheal lipopolysaccharide (LPS) administration, demonstrated impaired endothelium-dependent vasodilation of retinal arterioles. This dysfunction was associated with elevated generation of reactive oxygen species (ROS) and the prooxidative enzyme NOX2, indicating a critical role of oxidative stress in microvascular damage (Zadeh et al., 2019).

Interestingly, circulating GLP-1 levels are significantly increased in critically ill patients, as well as in those with cardiovascular events, suggesting that GLP-1 may counteract pro-inflammatory stimuli (Kahles et al., 2020; Lebherz et al., 2017). Laboratory studies have also indicated the potential of GLP-1 receptor agonists as therapeutic option in septic conditions (Yang et al., 2021). Although the exact mechanisms are not fully explored, the anti-inflammatory effects of GLP-1, as well as its potential to alleviate endothelial dysfunction independent of its glucose-lowering effects, are thought to be key contributors (Helmstädter et al., 2020; Kröller-Schön et al., 2012; Lee and Jun 2016). Animal models of sepsis are well-suited for studying inflammation, oxidative stress, and endothelial dysfunction (Steven et al., 2015). In a model of polymicrobial sepsis induced by cecal ligation and puncture (CLP), liraglutide treatment reduced oxidative stress and inflammation, improving sepsis-induced endothelial dysfunction in the aorta (Helmstädter et al., 2021).

However, there is a lack of studies examining the effects of GLP-1 receptor agonists on oxidative stress and endothelial dysfunction specifically in ocular blood vessels. Hence, this study aims to explore the antioxidant and endothelium-protective potential of liraglutide in ophthalmic arteries in mice with polymicrobial sepsis.

2. Materials and methods

2.1. Animals

All experimental procedures were carried out in strict adherence with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals, as recommended by the U.S. National Institutes of Health. The study received prior approval from the Ethics Committee of the University Hospital Mainz and the Landesuntersuchungsamt Rheinland-Pfalz (Koblenz, Germany; permit number: 23 177-07/G 14-1-039).

Male C57BL/6J mice, aged between 8 and 12 weeks, were acquired from Charles River. These mice were maintained in the institutional animal facility under controlled conditions, including a 12-h light/dark cycle, with unrestricted access to food and water.

The mice were assigned to one of three treatment groups: vehicle-treated sham-operated controls, vehicle-treated septic mice undergoing cecal ligation and puncture (CLP), and liraglutide-treated septic mice also receiving the CLP-procedure. Intraperitoneal liraglutide (100 μg/kg) or saline (vehicle with identic volume for sham-operated and septic mice) was administered one day prior to the CLP procedure. These injections were given twice daily at 12-h intervals and continued until the animals were killed.

The CLP procedure was performed as an experimental model to induce polymicrobial sepsis in rodents, following previously published protocols (Helmstädter et al., 2021). Briefly, mice were anesthetized using ketamine and xylazine (120 mg/kg and 16 mg/kg in 0.9 % NaCl). Through a midline laparotomy the cecum could be exposed, which was ligated afterwards below the ileocecal valve and punctured using a 21-gauge needle. A small amount of feces was located into the peritoneal cavity to promote bacterial peritonitis. Subsequently, the cecum was repositioned into the abdominal cavity, and the abdomen was closed with running sutures of the musculature and skin. Sham-operated animals underwent the identical procedures but without cecal ligation or puncture.

Following surgery, mice received subcutaneous injections of pre-warmed saline (37 °C, 50 mL/kg). Pain management was provided postoperatively by buprenorphine injections (0.05 mg/kg, s.c.) that were administered every 6 h. To ensure consistency of the procedure, all surgeries were conducted at the same time of the day and by the same surgeon. This CLP model is widely known to induce a clinically relevant scenario of polymicrobial sepsis, characterized by bacteremia, an excessive immune response and septic shock, with progression to multiorgan failure and death (Rittirsch et al., 2009).

At 48 h post-CLP, the animals were euthanized via cardiac puncture under anesthesia with ketamine and xylazine (120 mg/kg and 16 mg/kg in 0.9 % NaCl).

2.2. Preparation of ophthalmic arteries and measurement of vascular reactivity

After euthanasia, the eyes along with all retrobulbar structures were carefully removed and stored in cold Krebs buffer. A dissection microscope was used to isolate the ophthalmic artery, following previously published protocols (Birk et al., 2021; Buonfiglio et al., 2023; Manicam et al., 2017). In brief, the vessels were carefully dissected and cleaned of surrounding connective tissue using fine-tipped tweezers and micro-scissors. The vessels were then localized in an organ chamber containing cold Krebs solution, cannulated with borosilicate glass micropipettes, and fixed with 10-0 nylon monofilament sutures. A no-flow state with a pressure of 50 mmHg was generated through two compartments filled with Krebs buffer. The organ chamber was maintained at 37 °C, with a continuous circulation of oxygenated and carbonated Krebs solution adjusted to pH 7.4. The ophthalmic arteries were observed and recorded by a video camera attached to an inverted microscope. Before starting the experiments, the vessels were kept for equilibration over 60 min. The vessel's viability was confirmed by ensuring at least 50 % constriction from the baseline diameter in response to KCl (100 mM) depolarization. To assess contractile responses, the α_1-adrenoceptor agonist phenylephrine (10^{-8} to 10^{-3} M; Sigma-Aldrich, Steinheim, Germany) was applied, and its concentration-dependent effects on the vessel diameter were measured. By titration of phenylephrine, the same vessel segment was pre-constricted to at least 50 % of their native diameter. Concentration-response curves were generated for sodium nitroprusside (10^{-9} to 10^{-4} M), an endothelium-independent nitric oxide donor, and acetylcholine (10^{-9} to 10^{-4} M), an endothelium-dependent vasodilator, both from Sigma-Aldrich.

2.3. Quantification of reactive oxygen species

The generation of reactive oxygen species (ROS) was assessed in cryosections of the ophthalmic artery using fluorescence derived from dihydroethidium (DHE, 1 μM). Freshly isolated vessels were embedded in Tissue-Tek O.C.T. Compound (Sakura Finetek Europe, Alphen aan den Rijn, Netherlands) and rapidly preserved by freezing in liquid nitrogen. Cryosections of 10 μm thickness, were prepared using a Leica Reichert Jung 2030 cryostat (Leica, Rijswijk, The Netherlands) and mounted onto microscope slides (SuperFrost Plus™; Thermo Fisher Scientific, Waltham, MA, USA). After rinsing the slides in H_2O for 30 s to wash out the O.C.T. Compound, the prepared microscope slides with sections of the ophthalmic arteries were co-incubated with 1 μM dihydroethidium (DHE, Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C for 30 min. Vascular cross-sections were photographed under a fluorescence microscope, and the intensity of the red fluorescence signal was analyzed with ImageJ software (National Institutes of Health, Bethesda, MA, USA), performing densitometry, following previously described protocols (Birk et al., 2021; Dauth et al., 2023).

2.4. Quantification of prooxidative redox enzymes by real-time PCR

Messenger RNA expression levels for prooxidative redox enzymes, such as NOX1, NOX2, and NOX4, and for the inflammatory markers TNF-α and iNOS were determined in isolated ophthalmic arteries of vehicle-treated sham-operated mice, vehicle-treated septic mice, and liraglutide-treated septic mice. After euthanizing the mice and isolation of the ophthalmic arteries, the cleaned vessels were cut into small pieces, and placed in 1.5 ml tubes. After freezing the samples in liquid nitrogen, they were stored for 2–3 weeks at −80 °C,.

Subsequently, the tissue was homogenized in lysis buffer containing 1.0 % NP40, 0.5 % sodium deoxycholate, 0.1 % SDS, 10 mmol/L NaF, and 80 mmol/L TRIS (pH 7.5). A LightCycler LC480 (Roche Diagnostics, Mannheim, Germany) and a StepOnePlus system (Applied Biosystems, Foster City, CA, USA) were used to perform quantitative PCR (qPCR), following the manufacturer's instructions. The following cycling conditions were applied: initial denaturation (holding stage) for 10 min at 95 °C; amplification (cycling stage, 40 cycles) with 20 s at 95 °C and 40 s at 60 °C; and a melt curve stage starting at 60 °C, increasing by 0.5 °C every 5 s to 95 °C. To detect fluorescence of the amplified DNA, SYBR Green was applied. The comparative threshold (CT) method was conducted to quantify relative mRNA levels of the genes of interest, normalized to the β-actin gene (ACTB). The expression of mRNA is reported as the fold-change of vessels from vehicle-treated sham-operated mice. Primer sequences used for PCR are provided in Table 1.

2.5. Immunostainings

Cryosections of the ophthalmic arteries with a thickness of 10 μm were used for immunostainings. The tissue was fixed with paraformaldehyde (4 %) for 20 min and blocked for 30 min with bovine serum albumin (1 %). Next, the tissue sections were incubated with an antibody targeting NOX2 (ab129068, rabbit monoclonal, Abcam, Waltham, MA, USA, dilution 1:200) for 2 h at room temperature. Following incubation, the slides underwent three washes with PBS (5 min each) and were then exposed to a secondary antibody conjugated to Rhodamine Red-X (111-295-003, goat anti-rabbit polyclonal, Dianova GmbH, Hamburg, Germany; dilution 1:200) for 1 h at room temperature. To confirm the specificity of the primary antibody, tissue from NOX2−/− mice was used as a control. After washing the slides again in PBS (3 × 5 min), they were mounted with VECTASHIELD® Mounting Medium containing DAPI (BIOZOL Diagnostica Vertrieb GmbH, Eching, Germany), and finally covered with a glass-coverslip. Fluorescence intensity within the vascular wall of the ophthalmic arteries was quantified using Image J (NIH, http://rsb.info.nih.gov/ij/) as reported previously (Zadeh et al., 2019).

2.6. Statistical analysis

A one-way ANOVA and a Tukey's multiple comparisons test were applied for comparison of vascular diameters, fluorescence intensity, and mRNA expression levels. For comparison of concentration-response curves, two-way ANOVA and a Tukey's multiple comparison test were used. The level of significance was established at 0.05, with n denoting the number of mice in each group.

3. Results

3.1. Effects of liraglutide on vascular tone and endothelial function in septic mice

The initial luminal diameter of the ophthalmic artery assessed directly after cannulation was similar in vehicle-treated sham-operated mice (126 ± 12.40 μm), vehicle-treated septic mice (129 ± 14.86 μm) and in liraglutide-treated septic mice (137 ± 10.51 μm, $p > 0.05$). After equilibrating over 60 min, vessels of all three mouse groups developed spontaneous myogenic tone. With myogenic tone, luminal diameter did not differ significantly between vehicle-treated sham-operated mice (107 ± 12.65 μm), vehicle-treated septic mice (127 ± 18.96 μm) and liraglutide-treated septic mice (110 ± 6.951 μm, $p > 0.05$, Fig. 1).

Phenylephrine (10^{-8}-10^{-3} M) caused vasoconstriction in a concentration-dependent manner in vehicle-treated sham-operated mice, vehicle-treated septic mice and liraglutide-treated septic mice. No differences between the three groups were detected (Fig. 2A). Endothelium-independent vasodilatation induced by nitroprusside did not differ in vessels from all three mouse groups (Fig. 2B). In contrast, vehicle-treated septic mice showed significantly impaired responses to the endothelium-dependent vasodilator acetylcholine, indicative of endothelial dysfunction. However, liraglutide retained endothelial function in septic mice (Fig. 2C).

3.2. ROS levels in the ophthalmic artery

A significantly increased fluorescent intensity in the vascular wall of vehicle-treated septic mice in comparison to vehicle-treated sham-operated mice was detected by DHE staining of cross-sections of the ophthalmic arteries suggesting an elevated ROS generation under septic conditions (Fig. 3). Remarkably, excessive ROS generation in the ophthalmic artery was prevented by treatment of septic mice with liraglutide (Fig. 3).

Table 1
Primer sequences used for quantitative PCR analysis.

Gene	Accession Number	Forward	Reverse
ACTB	NM_007393.5	CACCCGCGAGCACAGCTTCTTT	AATACAGCCCGGGGAGCATC
NOX1	NM_172203.2	GGTTGGGGCTGAACATTTTTC	TCGACACACAGGAATCAGGAT
NOX2	NM_007807.5	GCACCTGCAGCCTGCCTGAATT	TTGTGTGGATGGCGGTGTGCA
NOX4	NM_015760.5	GGCTGGCCAACGAAGGGGTTAA	GAGGCTGCAGTTGAGGTTCAGGACA
TNF-α	NM_013693.3	GGCAGGTTCTGTCCCTTTCAC	TTCTGTGCTCATGGTGTCTTTTCT
iNOS	NM_010927.4	TTCACCCAGTTGTGCATCGACCTA	TCCATGGTCACCTCCAACACAAGA

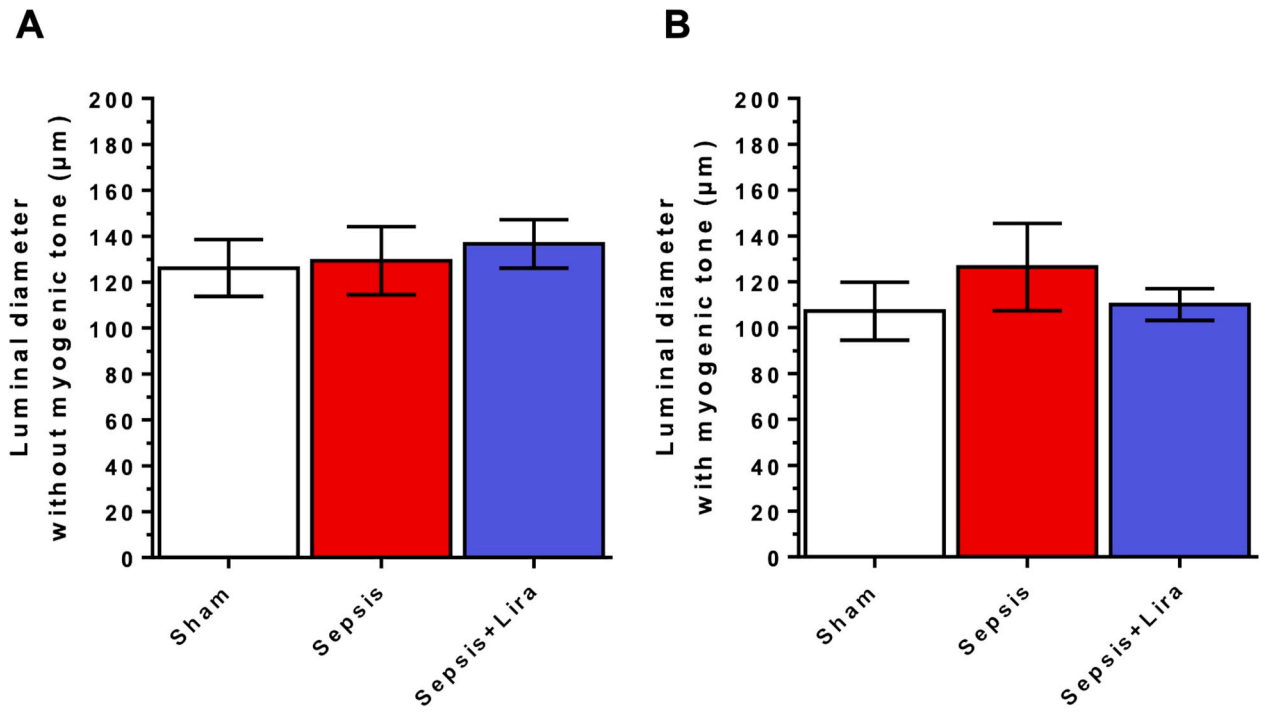

Fig. 1. Luminal ophthalmic artery diameter before (A) and after development of spontaneous myogenic tone (B) was similar in arteries of all three mouse groups. Myogenic tone was just by tendency weaker in arteries from septic mice (n = 6 per group).

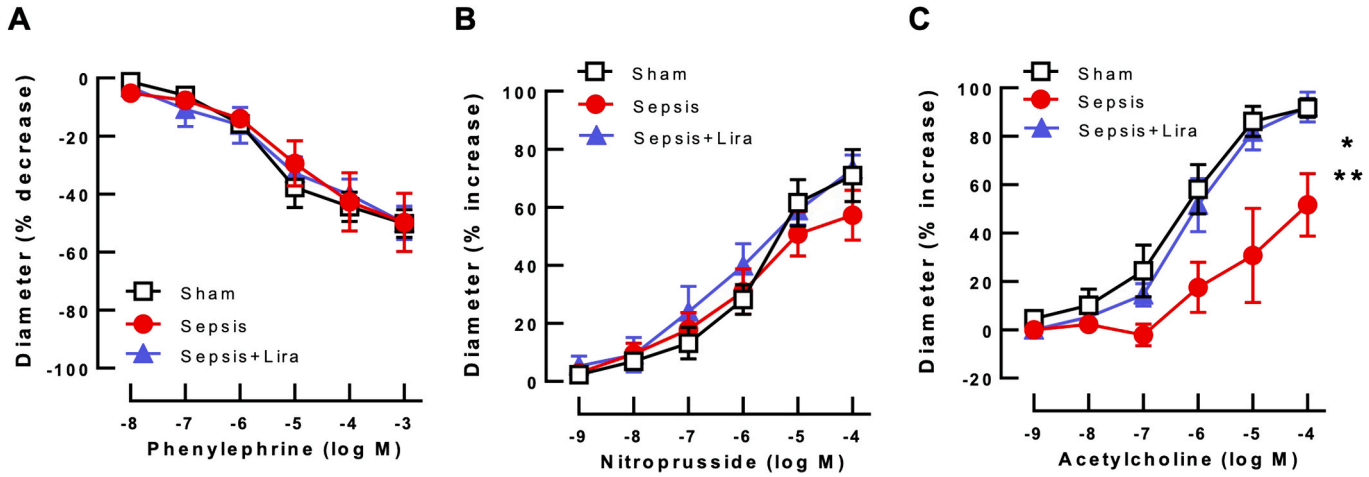

Fig. 2. The α_1-adrenoceptor agonist phenylephrine (A) produced similar constriction responses in vehicle-treated sham-operated mice (sham), vehicle-treated septic mice (sepsis), and liraglutide-treated septic mice (sepsis + lira). Likewise, the endothelium-independent vasodilator nitroprusside (B) elicited vasodilatory responses that were similar in vessels of all three mouse groups. In contrast, responses to the endothelium-dependent vasodilator acetylcholine (C) were reduced in vehicle-treated septic mice, indicative of endothelial dysfunction. Remarkably, liraglutide prevented endothelial dysfunction (*$p < 0.05$, sepsis vs. sepsis + lira; **$p < 0.01$, sham vs. sepsis + lira; n = 6 per concentration and group).

3.3. Messenger RNA expression for redox and inflammatory markers

The mRNA expression of the analyzed NOX isoforms (NOX1, NOX2, and NOX4) showed no differences between ophthalmic arteries of the three mouse groups. Remarkably, NOX1 mRNA was absent in the ophthalmic arteries across all three mouse groups. Expression of NOX2 showed a tendency towards higher expression in vehicle-treated septic mice, but without statistical significance. Expression of TNF-α and inducible NOS (iNOS) did also not differ between vehicle-treated sham-operated mice, vehicle-treated septic mice and liraglutide-treated septic mice (Fig. 4).

3.4. Expression of NOX2 in the vascular wall

Expression of NOX2 was tested on the protein level in cryo-sections of ophthalmic arteries using a specific antibody. Remarkably, pronounced NOX2 expression was observed especially in the endothelium and directly beneath the endothelium in ophthalmic arteries of vehicle-treated septic mice, but not in vehicle-treated controls or in liraglutide-treated septic mice (Fig. 5), indicating that liraglutide prevents sepsis-induced NOX2 expression.

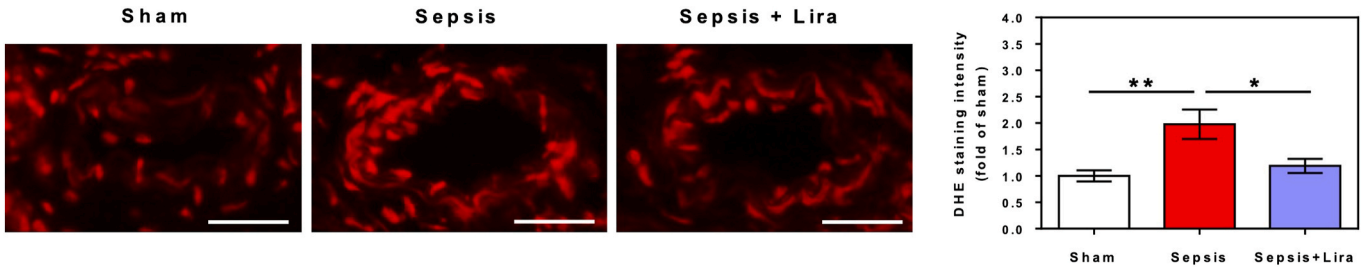

Fig. 3. Representative pictures of DHE-stained ophthalmic artery cross-sections from vehicle-treated sham-operated mice (sham), vehicle-treated septic mice (sepsis), and liraglutide-treated septic mice (sepsis + lira). Scale bar = 50 μm. Ophthalmic arteries of the sepsis group were characterized by increased DHE staining intensity in the vascular wall, indicative of elevated ROS levels. However, liraglutide prevented arteries of septic mice from excessive ROS generation (*p < 0.05; **p < 0.01, n = 6 per group).

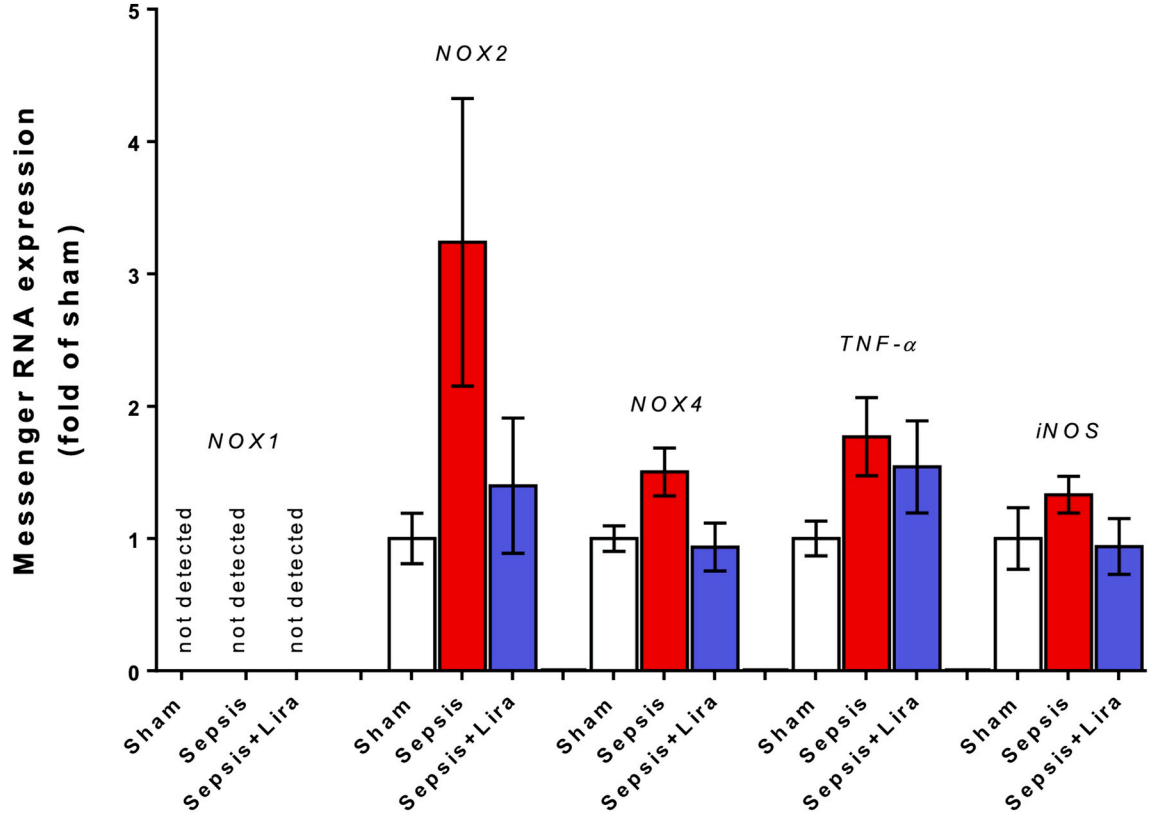

Fig. 4. While *NOX1* mRNA was not detectable in ophthalmic arteries of any of the mouse groups, *NOX2* mRNA expression was by tendency elevated in vehicle-treated septic mice. Also, no significant difference in mRNA expression levels across the groups was detected for *NOX4*, *TNF-α*, and *iNOS* (n = 6 per group).

4. Discussion

This is the first study in a murine model of polymicrobial sepsis, yielding several significant findings on ocular vascular reactivity and the therapeutic potential of liraglutide. First, endothelium-dependent vasodilation in ophthalmic arteries from vehicle-treated septic mice was markedly impaired, suggesting that polymicrobial sepsis induces endothelial dysfunction. Strikingly, liraglutide retained endothelial function in septic mice. As the underlying mechanism triggering endothelial dysfunction in sepsis, we identified oxidative stress from the prooxidative enzyme NOX2. Treatment with liraglutide significantly reduced ROS levels, suggesting that GLP-1 receptor agonists possess antioxidant properties.

Several laboratory studies in animal models have delved into the potential of GLP-1 receptor agonists to counteract endothelial dysfunction. For example, in mice with angiotensin II-induced arterial hypertension, the GLP-1 receptor agonist liraglutide was shown to exert cardio- and vasoprotective effects (Helmstädter et al., 2020). Oxidative stress was mitigated by reduction of endothelial leukocyte rolling and by inhibiting the infiltration of the vascular wall by myeloid cells. Additionally, liraglutide improved nitric oxide (NO) bioavailability by reducing eNOS uncoupling. These beneficial effects were lost in mouse models with global and endothelial cell-specific knockout of the GLP-1 receptor (Helmstädter et al., 2020). In the same mouse model and study design, as used in this study, functional vascular studies on the murine aorta demonstrated that sepsis induced by CLP, impaired endothelium-dependent relaxation of the aorta, while treatment with liraglutide ameliorated endothelial dysfunction. In addition, liraglutide treatment reversed elevated expression of pro-inflammatory cytokines, such as interleukin-6 (IL-6), TNF-α, but also the level of intercellular adhesion molecule-1 (ICAM-1) in the aortic wall. A tendency of reduced oxidative stress markers, including NOX2 expression and oxidative nitration modifications of proteins, was also demonstrated (Helmstädter et al., 2021). Similarly, liraglutide reduced inflammation and improved

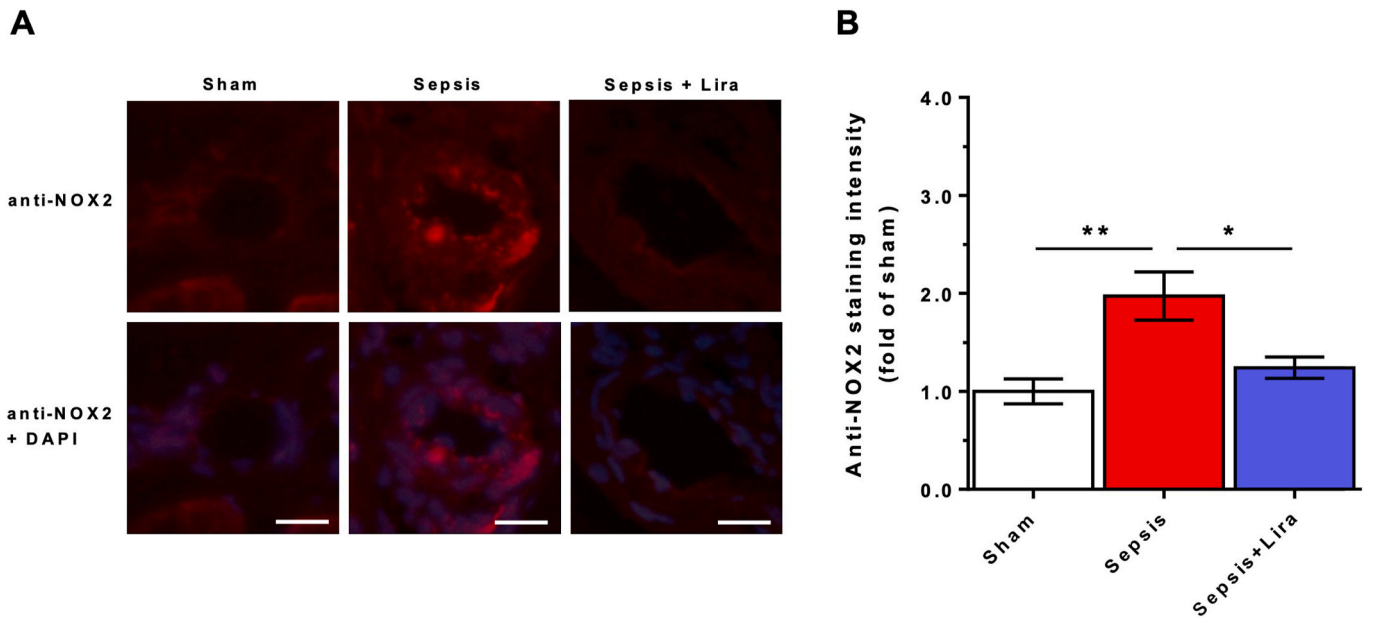

Fig. 5. (A) Representative pictures of anti-NOX2-stained ophthalmic artery cross-sections from vehicle-treated sham-operated mice (sham), vehicle-treated septic mice (sepsis), and liraglutide-treated septic mice (sepsis + lira). While immunoreactivity to NOX2 was only weak in ophthalmic arteries from vehicle-treated sham-operated mice and liraglutide-treated septic mice, pronounced immunoreactivity to NOX2 was seen especially in the endothelium vehicle-treated septic mice. Scale bar = 50 μm. (B) Immunoreactivity to NOX2 was elevated by around 2-fold in ophthalmic arteries from vehicle-treated septic mice compared to the other two groups (*$p < 0.05$; **$p < 0.01$, $n = 6$ per group).

aortic vascular function in rats with endotoxic shock induced by lipopolysaccharide (LPS) injection (Steven et al., 2015). Inhibitors of DPP-4, which increase GLP-1 bioavailability, or DPP-4 knockout models, showed comparable results, with improved vascular function, reduced inflammatory parameters, and enhanced survival of endotoxemic animals (Steven et al., 2015). In this same endotoxemia model, treatment with linagliptin, a DPP-4 inhibitor, or liraglutide reversed leukocyte-derived oxidative burst, markers for nitrosative stress, such as nitrosyl-iron hemoglobin, and the expression of iNOS. Furthermore, microvascular thrombosis was prevented through inhibition of platelet activation (Steven et al., 2017). The underlying mechanism likely involves GLP-1 receptor-mediated increase of cAMP levels with downstream activation of protein kinase A (PKA) (Steven et al., 2017).

Septic mouse models share several pathophysiological mechanisms with diabetic microvascular complications, especially with regard to oxidative stress and inflammation causing endothelial dysfunction. Protective effects of GLP-1 receptor analogs for vascular function and cardiovascular outcomes have been demonstrated in both experimental and clinical studies. A central scientific question is whether GLP-1 analogs can preserve vascular function and mitigate oxidative stress and inflammation independently of glycemic control. Treatment with GLP-1 receptor agonists has been demonstrated to offer vascular benefits in both patients with and without T2DM (Hernandez et al., 2018; Marso et al., 2016b; Wang et al., 2024). Beyond their glucose- and weight-lowering effects, these agents exhibit anti-inflammatory and antioxidant properties. These effects include the downregulation of pro-inflammatory cytokines and adhesion molecules, the inhibition of the NLRP3 inflammasome, promotion of NO synthesis in endothelial cells, preservation of mitochondrial functions, reduction of ROS generation, decreased macrophage infiltration and formation of foam cells, and improved vascular smooth muscle cell function (Wang et al., 2024). Several in vitro studies in non-diabetic animal models revealed that GLP-1 receptor activation in vascular endothelial cells promotes eNOS activation, resulting in enhanced synthesis of NO and suppression of NF-κB. These effects are controlled through various signaling pathways, notably the cAMP-dependent AMPK and the PI3K/AKT pathway (Erdogdu et al., 2010; Tang et al., 2018; Wu et al., 2021). Remarkably, endothelial cell migration and proliferation could be enhanced by liraglutide, thereby facilitating endothelial restoration and endothelial tube formation (Xiao et al., 2020). Metabolic analyses conducted in the same mouse model and experimental setting as in our study could not demonstrate any differences in non-fasting blood glucose levels when comparing sham-treated septic mice and liraglutide-treated septic mice, highlighting potential glucose-independent effects of liraglutide. Additionally, liraglutide treatment did not prevent the body weight loss induced by sepsis in septic mice. Interestingly, liraglutide treatment significantly normalized the body temperature of septic mice and, to a lesser extent, improved white blood cell and platelet counts, suggesting direct anti-inflammatory effects of the GLP-1 receptor (Helmstädter et al., 2021).

In this study, we focused on the role of liraglutide in reducing oxidative stress as a trigger of endothelial dysfunction. Consistent with our findings, other authors have reported on the antioxidant effects of liraglutide, including inhibition of various ROS sources. Some studies have reported that liraglutide activates the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, helping to reduce ROS generation (Yan et al., 2022). A significant portion of ROS is generated by different isoforms of NOX, an enzyme complex located in cell membranes (Böhm et al., 2023). NOX expression can be activated via PKC stimulation under high glucose conditions. Liraglutide was shown to prevent this PKC-mediated activation of NOX, thereby reducing ROS generation (Baylan et al., 2022; Ding et al., 2019; Inoue et al., 2015). Additionally, inhibitory effects of liraglutide on the receptor for advanced glycation end-products (RAGE) have been discussed (Ji et al., 2022).

This study revealed, that NOX2 expression was elevated especially in the endothelium and subendothelial space of ophthalmic arteries from septic mice, suggesting that NOX2 contributed to ROS generation and endothelial dysfunction. We previously observed impaired endothelial functions in retinal arterioles in a pig model of ARDS, induced by intratracheal LPS-administration, which was accompanied by NOX2 overexpression (Zadeh et al., 2019). Further studies in endotoxemic mice also identified a central role of NOX2 during retinal vascular inflammation, since knockout of NOX2 or treatment with aponycin, a NOX2 inhibitor, prevented ICAM-1 expression, leukostasis and

break-down of the blood-retinal barrier (Al-Shabrawey et al., 2008). Comparable findings were reported in studies in other vascular beds, such as the aorta or pulmonary microvasculature (Gandhirajan et al., 2013; Gill et al., 2015; Menden et al., 2015). These results support the fact that NOX2 overexpression is a hallmark of sepsis in many vascular beds. In line with these previous observations, liraglutide treatment prevented NOX2 overexpression and ROS level elevation, while preserving endothelial function in ophthalmic arteries of septic mice.

This is the first study assessing the potential of liraglutide in the prevention of endothelial-dysfunction and oxidative stress in ophthalmic arteries of a murine model of polymicrobial sepsis. In the next step, further studies evaluating potential effects of GLP-1 receptor analogs in animal models with retinal vascular diseases are mandatory.

5. Conclusion

This study revealed impaired endothelium-dependent vascular functions in ophthalmic arteries of mice with CLP-induced polymicrobial sepsis. Increased vascular ROS generation with consequent oxidative stress may represent a major underlying mechanism. As source of ROS, we identified NOX2, elevated especially in the endothelium and subendothelial space. By treatment of these septic mice with the GLP-1 receptor agonist liraglutide, endothelial functions and ROS levels could be retained. These findings emphasize the high potential of liraglutide for the treatment of diseases, in which endothelial dysfunction is a central pathophysiologic component. Thereby, GLP-receptor agonists become an attractive treatment strategy for retinal vascular diseases, such as diabetic retinopathy.

CRediT authorship contribution statement

Elsa Wilma Böhm: Writing – original draft, Investigation, Formal analysis. **Wael Omran:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Jenia Kouchek Zadeh:** Writing – review & editing, Methodology, Investigation. **Tschingis Arad:** Writing – review & editing, Investigation, Formal analysis. **Norbert Pfeiffer:** Writing – review & editing, Resources, Funding acquisition. **Andreas Patzak:** Writing – review & editing, Resources, Formal analysis. **Matthias Oelze:** Writing – review & editing, Resources. **Andreas Daiber:** Investigation, Conceptualization, Writing – review & editing, Resources. **Johanna Helmstädter:** Writing – review & editing, Methodology, Investigation. **Sebastian Steven:** Writing – review & editing, Resources, Project administration, Investigation, Conceptualization. **Adrian Gericke:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Informed consent statement

Not applicable.

Institutional review board statement

The animal study was approved by the responsible regulatory authority (Landesuntersuchungsamt Rheinland-Pfalz, approval number: 23 177-07/G 14-1-039).

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Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

Data will be made available on request.

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