Rivoxaban improves vascular response in LPS-induced acute inflammation in experimental models

Armond Daci1,2, Lorenzo Da Dalt3, Rame Alaj4, Shpejtim Shurdhiqi4, Burim Neziri5, Rrahman Ferizi6, Giuseppe Danilo Norata3,7, Shaip Krasniqi2*

1 Department of Pharmacy, Faculty of Medicine, University of Prishtina, Prishtina, Kosovo, 2 Institute of Pharmacology and Toxicology, Faculty of Medicine, University of Prishtina, Prishtina, Kosovo, 3 Department of Excellence of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy, 4 Cardiovascular Surgery Clinic, University Clinical Center of Kosovo, Prishtina, Kosovo, 5 Institute of Pathophysiology, Faculty of Medicine, University of Prishtina, Prishtina, Kosovo, 6 Department of Premedical Courses-Biology, Faculty of Medicine, University of Prishtina, Prishtina, Kosovo, 7 Centro SISA per lo Studio dell’Aterosclerosi, Ospedale Bassini, Cinisello Balsamo, Italy

* shaip.krasniqi@uni-pr.edu

Abstract

Rivoxaban (RVX) was suggested to possess anti-inflammatory and vascular tone modulatory effects. The goal of this study was to investigate whether RVX impacts lipopolysaccharide (LPS)-induced acute vascular inflammatory response. Male rats were treated with 5 mg/kg RVX (oral gavage) followed by 10 mg/kg LPS i.p injection. Circulating levels of IL-6, MCP-1, VCAM-1, and ICAM-1 were measured in plasma 6 and 24 hours after LPS injection, while isolated aorta was used for gene expression analysis, immunohistochemistry, and vascular tone evaluation. RVX pre-treatment significantly reduced LPS mediated increase after 6h and 24h for IL-6 (4.4 ± 2.2 and 2.8 ± 1.7 fold), MCP-1 (1.4 ± 1.5 and 1.3 ± 1.4 fold), VCAM-1 (1.8 ± 2.0 and 1.7 ± 2.1 fold). A similar trend was observed in the aorta for iNOS (5.5 ± 3.3 and 3.3 ± 1.9 folds reduction, P < 0.01 and P < 0.001, respectively), VCAM-1 (1.3 ± 1.2 and 1.4 ± 1.3 fold reduction, P < 0.05), and MCP-1 (3.9 ± 2.2 and 1.9 ± 1.6 fold reduction, P < 0.01). Moreover, RVX pre-treatment, improved LPS-induced PE contractile dysfunction in aortic rings (Control vs LPS, Emax reduction = 35.4 and 31.19%, P < 0.01; Control vs LPS+RVX, Emax reduction = 10.83 and 11.48%, P > 0.05, respectively), resulting in 24.5% and 19.7% change in maximal constriction in LPS and LPS+RVX respectively. These data indicate that RVX pre-treatment attenuates LPS-induced acute vascular inflammation and contractile dysfunction.

Introduction

Coagulation plays a key role in cardiovascular disorders [1] and interfering with coagulation factors represents one of the main pharmacological approaches in CVD [2]. Coagulation factors, not only participate to the activation of the coagulation cascade but also impact vascular function; this is the case for factor X (FXa), one of the main components in the coagulation
process [3], which, through the activation of protease-activated receptors (PAR) [4], affects, vasomotor responses, inflammation, endothelial function, vascular proliferation, cellular hypertrophy, atherosclerosis, and thrombosis [5,6].

As hemostatic and inflammatory pathways are highly interconnected [7,8], the approval of novel oral anticoagulants (NOAC), affecting FXa activity and prothrombin complexes (rivaroxaban, apixaban, betrixaban, and edoxaban) [9], have raised interest in the interplay between haemostasis and inflammation linking FXa blockade to PAR inhibition [10,11], and potentially to improved vascular function.

Clinical studies with NOACs have shown that these drugs reduce the incidence of cardiovascular events including coronary and peripheral artery disease, cerebral ischemia, thrombosis, thromboembolic events, and atherosclerosis [12,13]. In addition to this, experimental studies proposed a series of vascular protective properties of NOAC via inhibition of FXa [14–26]. These include potential anti-inflammatory effects [15,19–21,23,24,27], that might perhaps impact vascular function and pathology [14,25]. Indeed inflammation is one of the main contributing factors in coronary artery disease leading to the development of atherosclerosis [28]. Moreover, acute exposure to lipopolysaccharide endotoxin (LPS) has been shown to induce an inflammatory response that in turn supports vascular injury and dysfunction [29,30].

This raises the intriguing possibility that the impact on NOACS on vasomotor function [17,31] might depend also on the ability to control vascular function under acute inflammatory conditions.

To this aim, we used isolated rat aorta, to test the hypothesis that pre-treatment with Rivaroxaban (RVX) might mitigate LPS-induced acute vascular inflammation with a focus on pro-inflammatory, pro-adhesive, and contractile responses under LPS-induced vascular inflammatory conditions.

**Material and methods**

**Animals and treatment**

Wistar rats between 10–12 weeks of age (220–260 g) were used in our study. All rats were fed with a normal chow diet during the period of our study. Animals were accommodated in normal rat cages with automatically controlled 12-hours light/12-hour dark cycle and the standard temperature-humidity environment with ad libitum water and food intake. Acute inflammation was induced by a single intraperitoneal (i.p) injection of LPS (10 mg/kg body weight) [32]. RVX (5 mg/kg body weight; supplied by Bayer Pharma AG) was administered via oral gavage 2 hours before LPS injection, the non-RVX groups (control and LPS only) received oral gavage of RVX vehicle (Carboxymethylcellulose Sodium 0.5%). RVX dose and interval used in our study was previously shown to inhibit the in vivo Factor Xa in rat arteriovenous shunt model [33] or thrombus formation [34] and was chosen based on previous in-vivo related mice and rat animal studies [14,17,35,36], which are specific in the previously reported single p.o administration of the RVX pharmacokinetic profile as well [37,38]. Our study protocol has been approved by the Ethical Committee of Medical Faculty–University of Prishtina (Nr. 4962), and all procedures for animal experiments were performed in compliance with guidelines for care and use of animals during whole experimentation procedures.

**ELISA**

Rats were sacrificed with an i.p overdose of sodium thiopental injection (50 mg/kg body weight) at 6 hours and 24 hours after LPS injection. Blood was collected from the left ventricle (EDTA containing tubes) and plasma isolated following centrifugation (4000 rpm for 10 minutes) and stored at -80°C. IL-6, MCP-1, VCAM-1, and ICAM-1 plasma levels were measured.
by enzyme-linked immunoassay kit (Abcam, Cambridge, MA) according to manufacturer’s protocol instructions.

**Aorta preparation**

After blood collection, the whole rat aorta was isolated and cleaned immediately from adhering perivascular adipose and connective tissues. Aortic rings of 4–6 mm were cut from the ascending aorta and fixed in 10% neutral buffered formalin for further immunohistochemical analysis. Subsequently, aortic rings of 5–7 mm were cut from the remaining part of the ascending aorta and descending aorta and snap-frozen in liquid nitrogen for gene expression analysis. Finally, aortic rings of 3–5 mm were cut from the remaining part of the thoracic aorta and used for testing vascular reactivity in the tissue organ bath.

**Immunohistochemistry**

Formalin-fixed aortic rings were embedded in paraffin and sectioned in 2.5 μm sections. Tissue sections were deparaffinized as described [39], rehydrated and the heat mediated antigens retrieval was performed by placing the slides in 10 mM sodium citrate buffer (pH 6.0) for 45 minutes at 95–98˚C. Blocking of endogenous peroxidase activity and non-specific staining was done with hydrogen peroxide and protein block. Subsequently, sections were incubated for 30 minutes with primary antibodies at the following dilution: 1:200 for anti-VCAM-1, 1:200 for anti-MCP-1, or 1:100 for Anti-iNOS. After washing steps, sections were incubated with a biotinylated secondary antibody (goat anti-polyvalent, Mouse, and Rabbit Specific HRP/DAB (ABC) Detection IHC kit, Abcam, Cambridge MA) for additional 15 minutes followed with streptavidin peroxidase 10’ incubation. Peroxidase activity was detected in fixed tissues with DAB substrate chromogen forDetection of HRP-conjugated antibody and followed under a microscope to determine staining development. Finally, after the tipping DAB and rinsing in water, the slides were counterstained with hematoxylin histological staining reagent as described [40]. Images were acquired with Olympus CX41 microscope (Olympus America) with Olympus SC100 Digital camera and cell Sens Imaging Software. Relative quantification of IHC staining has been done with Image J (NIH, https://imagej.nih.gov/ij/).

**Reverse transcription-quantitative real-time PCR (RT-qPCR) analysis**

Total RNA was isolated from aortae by using RNasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany) following the standard protocol. RNA assessed for quality and quantity using absorption measurements (NanoDrop™ 1000 Spectrophotometer, Thermo Fisher Scientific) and retro-transcribed in cDNA with iScript™ cDNA synthesis kit (BioRad) as described [41]. Gene expression analysis was performed using SYBR Green Supermix (Thermo Fisher Scientific) in CFX connect light cycler (BioRad, Cat#1708841) [42]. Expression was calculated using the ΔΔCt method (Livak and Schmittgen, 2001) and normalized to a housekeeping gene (GAPDH). The sequences are presented in S1 Table and expression levels were expressed with the fold change.

**Vascular tone**

Aortic ring fragments were mounted in the Tissue Organ Baths (750TOBS, DMT-USA, Ann Arbor, MI, USA) containing 10 mL of Krebs-Henseleit buffer (118.4 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM KH2PO4, 1.2 mM MgSO4, 25 mM NaHCO3, 11.1 mM glucose; pH 7.4). The temperature was adjusted at 37˚C and the buffer solution was bubble gassed with 5% CO2 and 95% O2 during the whole experiment. Changes in force tension were recorded by
isometric force-displacement transducers and continuous mode on a multichannel recorder polygraph model attached with software LabChart7 connected to power lab 4/35 data acquisition system (PowerLab 4/35, ADInstruments Pty Ltd., NSW, Australia).

Each ring was initially stretched to an optimal load (~2 g). Subsequently, preparations were equilibrated for 60 minutes with changes of fluid every 15 minutes. After the equilibration period, vessel specimen viability was tested with KCl (40 mM) induced contraction, and aortic segment preparations were washed until returning the basal tone. Thereafter, the vascular tone was determined with cumulative concentration-response curves with phenylephrine ($10^{-8}$ to $10^{-5}$ M).

**Statistical analysis**

All data are presented and calculated with mean ± SEM. The number of rats used in our study was expressed with “n”. A comparison of parameters obtained within the analysis between two groups was performed with unpaired Student’s t-test. Vasoreactivity of the PE contractions was calculated as a percentage of the KCl (40 mM) maximal initial contraction value. The concentration-response curve values were analyzed with two-way ANOVA followed by Tukey’s post hoc test for comparison between groups. P-value < 0.05 was considered to represent a statistically significant group difference. All analyses and graphs were performed using GraphPad PRISM version (6.0).

**Results**

3.1 Effects of RVX pre-treatment on the acute LPS-induced increase in IL-6, MCP-1, VCAM-1 and ICAM-1 levels

In order to investigate the impact of RVX pre-treatment on LPS-induced pro-inflammatory and proadhesive mediators expression, IL-6, MCP-1, VCAM-1, and ICAM-1 levels were measured in plasma 6 hours and 24 hours post LPS injection. As expected, LPS injection increased plasma levels IL-6, MCP-1, VCAM-1, and ICAM-1 (P < 0.001) (Fig 1A–1D); an effect which was significantly blunted with RVX pre-treatment (5 mg/kg) for IL-6 (P < 0.01), MCP-1 (P < 0.05) and VCAM-1 (P < 0.05) both after 6 and 24 hours of treatment. These results suggest that RVX pretreatment limits LPS induced inflammatory response (Fig 1A–1C).

3.2 Effects of RVX pre-treatment on IL-6, MCP-1, VCAM-1 and ICAM-1 gene expression the aorta

Next, we tested whether RVX treatment could improve pro-inflammatory gene expression in the aorta and liver. RVX pre-treatment (5 mg/kg) attenuated IL-6 and MCP-1 mRNA expression in the arterial wall (Fig 2A and 2B), while non-significant changes were observed for VCAM-1 and ICAM-1 expression at this site. Most importantly RVX pre-treatment did not affect LPS induced liver expression of IL-6, MCP-1, VCAM-1, and ICAM-1, by confirming also the liver as a key target of LPS induced acute inflammation (S1 Fig).

3.3 Effects of RVX pre-treatment on LPS-induced iNOS, MCP-1, and VCAM-1 wall expression in the vascular wall

Increased iNOS, MCP-1, and VCAM-1 immunoreactivity were observed in the aortic vascular tissues of LPS treated rats mainly in vascular endothelium, and the subendothelial layer was characterized by smooth muscle cells and perivascular adipose tissues compared to controls (P < 0.001) (Fig 3A–3C). RVX pre-treatment (5 mg/kg) reduced LPS-induced iNOS, MCP-1, and VCAM-1 expression both at 6 h and 24 h following LPS injection (Fig 3A–3C).
3.4 Effects of RVX pre-treatment on the acute LPS-induced PE contractile dysfunction in aortic rings

Next, we addressed whether improved anti-inflammatory effects of RVX pre-treatment (5 mg/kg) translate into the amelioration of LPS-induced contractile dysfunction to PE. LPS injection deteriorated PE-induced vasoconstriction when compared with the control group [Emax, 62.33±3.8% for LPS (6 h) compared to controls: 97.70±2.30%, P < 0.001; and Emax, 71.91±4.81% for LPS (24 h) compared to controls: Emax, 103.1±3.61%, P < 0.01] (Control vs LPS, Emax reduction = 35.4 and 31.19%, P < 0.001) (Fig 4A and 4B) (Table 1).

RVX pre-treatment (5 mg/kg) partially reverted LPS-induced PE contractile dysfunction at both 6 h and 24 h following LPS injection (Emax, 86.87±2.72%, and 91.62±5.83%, for LPS +RVX treatment; vs Emax, 62.33±3.8 and 71.91±4.81 for LPS alone, P < 0.01), maximal constriction with RVX+LPS was 24.5 and 19.7% higher compared to LPS alone 6 h and 24 h respectively (Fig 4A and 4B) (Table 1). These results suggest that RVX attenuates contractile dysfunction to PE during acute LPS inflammation.

Fig 1. Role of RVX pre-treatment on LPS-induced proinflammatory and proadhesive mediator release in rat plasma. IL-6 (A), MCP-1 (B), VCAM-1 (C) and ICAM-1 (D) plasma levels (pg/mL) from LPS treated rats in the presence or absence of RVX for 6 hours and 24 hours compared to controls. ###P < 0.01 and ##P < 0.001 (Student’s t-test) vs. control conditions. * P < 0.05, ** P < 0.01 and *** P < 0.001 (Student’s t-test) vs LPS. Values are expressed as the mean±SEM (n = 6).

https://doi.org/10.1371/journal.pone.0240669.g001

Fig 2. Role of RVX pre-treatment on LPS induced proinflammatory and proadhesive mediator’s gene expression in rat aorta. Comparisons of relative IL-6, MCP-1, VCAM-1 and ICAM-1 gene expression levels normalized to GAPDH in the rat aorta obtained from RVX- or non-treated LPS rats and non-treated control rats 6 hours (A) and 24 hours (B) post LPS exposure. * P < 0.05, ** P < 0.01 and *** P < 0.001 indicates values significantly different (Student’s t-test) vs LPS. Values are expressed as the mean±SEM (n = 6).

https://doi.org/10.1371/journal.pone.0240669.g002
Discussion

In this study, we demonstrated that a specific inhibitor of FXa, namely rivaroxaban, improves acute inflammation and vascular dysfunction following LPS-induced endotoxin shock.

Besides the role of factor Xa in the coagulation process, this factor contributes also to the pathogenesis of cardiovascular inflammatory disease through PARs and non-PAR receptors signaling mediated response in the vasculature [43,44]. Moreover, previous studies have shown that LPS affects the coagulation cascade by targeting FXa and its intracellular signaling which contributed to the increased inflammatory response and vascular modulation mainly through PAR activated receptors signaling [6,45,46]. For instance, the PAR-2 signaling activation contributes to the activation of macrophages and also to vascular inflammation [47]. Interestingly the time-dependent activation of PAR-2 receptors in the vascular and respiratory
tissues obtained from rats is induced from LPS itself [48,49]. Also, in other studies, these response was followed by the activation of the inflammatory pathway, via TL-4/NF-κB signaling [50,51]. Most of these responses were shown to be with RVX (Table 2). Of note LPS causes an inflammatory state characterized by increased proinflammatory and pro adhesive responses [52,53], and this could propagate in septic shock and related major complications such as organ failures e.g. respiratory, heart or kidney failures, or abnormal blood clotting (DIC) [54].

Nowadays, there are different experimental and clinical therapeutic interventions in sepsis [55–57], and targeting the cross-talk between inflammation and coagulation represents an emerging approach for targeting acute conditions as well as improving long term vascular outcomes in inflamed conditions [8,58,59].

Most clinical studies demonstrated that targeting Factor Xa inhibition with NOAC including rivaroxaban prevented systemic thromboembolic disease, reduced cardiovascular events, and death [60]. Moreover, NOAC non-hemostatic cellular effects suggest a potential benefit in inflammation, arterial stiffness, neointima formation, atherosclerosis, and fibrosis [5,61].

Although some anti-inflammatory effects, improvement of hypercoagulable actions such as disseminated intravascular coagulation (DIC), additional acute lung injury from endotoxemia [19,36,50], and additional vasculoprotective properties of RVX have been proposed in different in vitro and tissue models [14,15,20,21,23–25] (see Table 2), a beneficial effect on LPS induced acute vascular inflammatory response in vivo was not investigated yet. In murine macrophages and human tubular cells stimulated with FXa, RVX treatment was shown to reduce the expression of TNF-α, IL-1β, and MCP-1 [14,62]. Similarly, RVX dampened the expression of VCAM-1, ICAM-1, MCP-1, IL-8, CXCL1, CXCL2, TF in thrombin stimulated human endothelial cells [19], as well as IL-6, IL-1β, TNF-α, MMP9, and COL-1 expression in hypoxic cardiac myocytes and fibroblasts [23]. Similarly, also TNF-α, MCP-1, IL-6 expression in angiotensin II-induced inflammatory response in human podocytes was modulated by RVX [24].

Herein, our results extend these findings by demonstrating in vivo that RVX pre-treatment decreased the expression of pro-inflammatory mediators and adhesion molecules namely IL-6, MCP-1, and VCAM-1 induced by LPS in the aorta.

Table 1. Role of RVX on the vasoreactivity of aortic rings obtained from rats sacrificed 6 h and 24 h post LPS to PE-induced dose dependent contractions.

| Contractile Agent | Pretreatment | pEC50 | Emax | N |
|-------------------|--------------|-------|------|---|
| Control 6 h       | 7.06±0.06    | 97.70±2.30 | 6   |
| PE LPS 6 h        | 5.92±0.11+++ | 62.33±3.80+++ | 6   |
| LPS+RVX 6 h       | 6.68±0.06$  | 86.87±2.72## | 6   |
| Control 24 h      | 6.96±0.06    | 103.1±3.61 | 6   |
| LPS 24 h          | 6.28±0.12++  | 71.91±4.8#  | 6   |
| LPS+RVX 24 h      | 6.74±0.08$  | 91.62±5.8#  | 6   |

PE: Phenylephrine. Values are mean ± SEM from (n) different patients. pEC50 and Emax (maximal contraction, % KCl 40 mM) are derived from concentration-response curves presented in Fig 4A and 4B. These values are significantly different:

** P<0.01
+++ P<0.001 vs corresponding controls (Control)
# p<0.05
## p<0.01 vs corresponding controls (LPS 6 h)
+++ p<0.001
++ p<0.01
$ $ p<0.01
$ p<0.05, when compared to pEC50 values derived from corresponding controls vasoconstriction.

https://doi.org/10.1371/journal.pone.0240669.t001
Table 2. Basic experimental studies that investigate the anti-inflammatory properties of pre-treatment and post-treatment with RVX.

| Species       | Tissue/Model                  | Pre-Treatment (1) | Post-Treatment (2) | Response 1                                                                 | Response 2                                                                 | References |
|---------------|-------------------------------|-------------------|-------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|------------|
| Rat           | Lung/LPS                      | RVX               | -                 | TNF-α, MCP-1, IL-1β, PAR-2, NF-κB ↓                                        | -                                                                         | [50]       |
| Rat           | Femoral Artery/Atherosclerosis Obliterans | -                 | RVX               | IL-1, MCP-1, TNF-α, NF-κB/TLR4 ↓                                         | -                                                                         | [51]       |
| Rat           | Middle Cerebral Artery/ Temporary focal cerebral ischaemia | RVX               | RVX               | IL-1β, IFN-γ, TNF-α, ICAM-1, CD68 ↓                                       | -                                                                         | [26]       |
| Mice          | Aorta/ApoE +/-                | -                 | RVX               | TNF-α, IL-6, MCP-1, Egr-1, IFN-γ ↓                                       | -                                                                         | [35]       |
| Mice          | Aorta/ApoE +/-                | -                 | RVX               | TNF-α, COX-2, iNOS, MMP-9, MMP-1 ↓                                       | -                                                                         | [14]       |
| Mice          | Aorta/ApoE +/-                | -                 | RVX               | PAR-1, PAR-2, Mac-2, MMP-9 ↓                                            | -                                                                         | [63]       |
| Mice          | EJV/Catheter Thrombosis       | RVX               | -                 | MCP-1, MMP-9 ↓                                                           | -                                                                         | [15]       |
| Mice          | Atrial/TAC                    | -                 | RVX               | TNF-α, MCP-1, IL-1β, IL-6, PAR-2 ↓                                       | -                                                                         | [21]       |
| Mice          | Left Ventricle/TAC            | RVX               | -                 | IL-1β, IL-6, IFN-γ, NF-κB, TGF-β, CD-45 ↓                                 | -                                                                         | [77]       |
| Mice          | Left Ventricular/Myocardial Ischaemia-R1 and TF | RVX               | -                 | IL-6, PAR-2, collagen 1α2 and 3α1 ↓                                       | -                                                                         | [16]       |
| Mice          | Heart/Myocardial Infarction   | -                 | 138.5mg/kg/day    | TNF-α, PPAR-1, PAR-2, TGF-β, ↓                                           | -                                                                         | [78]       |
| Mice          | Aortic root, Coronary Arteries/ ICM | -                 | RVX               | IL-1β, IL-6, NF-κB                                                      | -                                                                         | [23]       |
| Mice          | Kidney/Ren-TG Hypertensive    | -                 | RVX               | TNF-α, MCP-1, Pal-1, PAR-2 ↓                                            | -                                                                         | [24]       |
| Mice          | Lung/BERK⁺⁺, vascular Inflammation | -                 | RVX               | IL-6, MPO, TAT ↓                                                         | -                                                                         | [64]       |

(Continued)
Earlier studies in ApoE-deficient mice showed that chronic administration of RVX reduced gene and protein expression for IL-6, TNF-α, MCP-1, i-NOS, COX-2, MMP9 in thoracic and abdominal aortas, attenuated macrophage activation, necrotic core formation, collagen loss, and promoted the stabilization of the atherosclerotic plaque [14,35,63].

Interestingly, a recent study has shown a cardioprotective effect of RVX pre-treatment in ischaemic cardiomyopathy in mice model with diet-induced myocardial infarction [23]. This study has shown that attenuation of cardiac remodeling, fibrosis, alleviation of the aortic root and coronary arteries atherosclerosis is dependent on the reduction of IL-1β, TNF-α, IL-6 cardiac mRNA expression, and nuclear factor kappa B (NF-κB) activation pathway in RVX pre-treated group. Also in the myocardial reperfusion injury mice model, RVX improved survival rates, cardiac function, and reduced IL-6, collagen 1α2, and 3α1 cardiac mRNA expression [16]. RVX protective effects were shown also in a rat model of brain ischemia/reperfusion injury where it reduced VCAM-1 protein expression, macrophage activation, and thrombin

Table 2. (Continued)

| Species   | Tissue/Model                  | Pre-Treatment (1) | Post-Treatment (2) | Response 1          | Response 2          | References |
|-----------|-------------------------------|------------------|-------------------|---------------------|---------------------|------------|
| Mice      | Hind Limb/STZ Diabetes, Ischaemia | RVX 1 or 3 mg/kg/day | RVX 1 or 3 mg/kg/day | -                   | Neovascularisation, CD-31, VEGF ↑ | [17]      |
|           |                               | 2 weeks          | 3 weeks           |                     |                     |            |
| Mice      | Femoral Arteries/Wire-Mediated Vascular Injury | RVX 5 mg/kg/day | RVX 5 mg/kg/day | -                   | TNF-α, MCP-1, IL-1β, (TGF)-β1, SDF-1, GM-CSF ↓ | [25]      |
| Human     | HUVEC/Thrombin                | RVX 0.3–3000 nM 30 min | -                | ICAM-1, ELAM-1, IL-8, MCP-1, CXCL1, CXCL2, TF ↓ | -            | [19]      |
| Human     | HUVEC/Inflammation            | RVX 1000 nM 24 hours | -                | TNF-α, IL-6, IL-1β, NF-κB ↓ | -            | [79]      |
| Human     | HUVEC/FXa Inflammation        | -                | RVX 50 nM 12 h    | -                   | CCL-2,CCL-5, EDN2, ITGAS, SELE, VCAM-1, TNSF10, MMP-2 ↓ | [80]      |
| Human     | Abdominal Aorta/Aneurysm      | -                | RVX 50 nM         | -                   | IL-6, NOS-2, MMP-9 ↓ | [20]      |
| Human     | Podocytes/Ang-II-induced Inflammation | RVX 500 μg/L 1 hour | -                | -                   | TNF-α, MCP-1, IL-6, PAR-2, NF-κB ↓ | [24]      |
| Human     | Kidney Tubular Cells/AGEs     | -                | RVX 300 nM 4 hours | -                   | MCP-1, ↓ | [62]      |
mediated thrombus formation [26], and also in pressure overload-induced atrial remodeling with transverse aortic constriction mice model where a reduced macrophage infiltration associated to a decreased expression of MCP-1, IL-6, IL-1β, TNF-α was observed [21].

Moreover, additional studies showed that RVX pre-treatment prevented the development of mechanical femoral vascular injury-induced neointima hyperplasia in mice, again by affecting IL-1β and TNF-α gene expression [25]. Similarly, RVX treatment decreased MCP-1 plasma levels and MMP-9 protein levels in the external jugular vein of mice following catheter thrombosis [15], as well as IL-6 plasma level and neutrophil levels in a mouse model of sickle cell disease [64]. Incubation, ex-vivo, of human abdominal aortic aneurysmal tissues, resulted in the reduction of IL-6 release and NOS-2, MMP9 protein expression [20].

Also, hypertensive renal damage resulted to be ameliorated by RVX chronic pre-treatment of renin overexpressing mice via specifically targeting of TNF-α, MCP-1, and IL-6 [24]. Thus paving the way also to the other newer FXa inhibitor, which recently demonstrated to affect VCAM-1 and ICAM-1 in uremia induced vascular dysfunction [65].

In addition to vascular inflammation, the acute inflammatory response from LPS induces vascular hyporeactivity and hypotension which were shown to be also time-dependent [66,67], thus displaying the highest level of vascular hyperresponsiveness and iNOS expression 6 hours post-exposure to LPS injection [32,68], as observed in our study experimental model. Moreover, FXa has been found to induce hypotension and inflammation response in vascular endothelial cells [43,44], whereas endotoxin activation of FXa and its intracellular signaling have been shown to trigger vascular tone reduction and hypotension [49,69–71]. This effect was shown to depend on factor Xa induced dilation of the rat aorta through the PAR-2 signaling pathway, a contribute pathway which was implicated also in severe hypotension following septic shock [72].

In this study, we demonstrate that RVX pre-treatment improves aortic hyporesponsiveness to PE under inflammatory conditions (Fig 5).
Previous studies tested in vitro the protective role of FXa inhibitors on the vascular tone of control rat aorta [73,74], mesenteric and basilar arteries [31], and streptozotocin-induced diabetic mice [17,75]. We now translate these findings in vivo by showing an improvement of vascular tone in endotoxin-induced hypotension and proinflammatory response following RVX pre-treatment. The effect could rely on the control of FXa-PAR-2 [47–49,69,76] and TL-4 /NF-κB signaling [24,50,51,77].

Although future additional studies are needed to better delineate the mechanisms beyond these effects, the currently available findings set the stage for investigating the additional molecular effects and also clinical benefit of RVX treatment in inflammation and hypotension associated with endotoxin shock.

Supporting information

S1 Fig. Role of RVX pre-treatment on LPS induced proinflammatory and proadhesive mediator’s gene expression in rat liver. Comparisons of relative IL-6, MCP-1, VCAM-1 and ICAM-1 gene expression levels normalized to GAPDH in the rat liver samples obtained from RVX- or non-treated LPS rats and non-treated control rats. *P<0.05, ** P<0.01 and ***P<0.001 indicates values significantly different (Student’s t-test) vs LPS. Values are expressed as the mean±SEM (n = 6).

(TIF)

S1 Table. Primer sequences.

(DOCX)

Author Contributions

Conceptualization: Armond Daci, Giuseppe Danilo Norata, Shaip Krasniqi.

Data curation: Armond Daci, Lorenzo Da Dalt, Burim Neziri, Rrahman Ferizi, Giuseppe Danilo Norata.

Formal analysis: Rrahman Ferizi.

Investigation: Armond Daci, Lorenzo Da Dalt, Rame Alaj, Shpejtim Shurdhiqi, Burim Neziri, Rrahman Ferizi, Giuseppe Danilo Norata.

Methodology: Armond Daci, Giuseppe Danilo Norata.

Project administration: Shaip Krasniqi.

Resources: Armond Daci, Rrahman Ferizi.

Supervision: Giuseppe Danilo Norata, Shaip Krasniqi.

Visualization: Burim Neziri, Rrahman Ferizi.

Writing – original draft: Armond Daci.

Writing – review & editing: Armond Daci, Giuseppe Danilo Norata, Shaip Krasniqi.

References

1. Olie RH, van der Meijden PEJ, ten Cate H. The coagulation system in atherothrombosis: Implications for new therapeutic strategies. Res Pract Thromb Haemost. 2018; 2: 188–198. https://doi.org/10.1002/rth2.12080 PMID: 30046721

2. Weitz JI, Fredenburgh JC. Factors XI and XII as targets for new anticoagulants. Frontiers in Medicine. Frontiers Media S.A.; 2017. https://doi.org/10.3389/fmed.2017.00019 PMID: 28286749
3. Rupprecht HJ, Blank R. Clinical pharmacology of direct and indirect factor xa inhibitors. Drugs. 2010. pp. 2153–2170. https://doi.org/10.2165/11538030-00000000-00000 PMID: 20964458

4. Gieseler F, Ungefoeren H, Settmacher U, Hollenberg MD, Kaufmann R. Proteinase-activated receptors (PARs)—Focus on receptor-receptor-interactions and their physiological and pathophysiological impact. Cell Communication and Signaling. 2013. https://doi.org/10.1186/1478-811X-11-86 PMID: 24215724

5. Papadaki S, Tselepis AD. Nonhemostatic Activities of Factor Xa: Are There Pleiotropic Effects of Anti-FXa Direct Oral Anticoagulants? Angiology. SAGE Publications Inc.; 2019. pp. 896–907. https://doi.org/10.1177/0003319719840861 PMID: 31010298

6. Ebrahimi S, Rezaei S, Seiri P, Ryzhikov M, Hashemy SI, Hassanian SM. Factor Xa Signaling Contributes to the Pathogenesis of Inflammatory Diseases. J Cell Physiol. 2017; 232: 1966–1970. https://doi.org/10.1002/jcp.25714 PMID: 27925197

7. Esmon CT. The interactions between inflammation and coagulation. Br J Haematol. 2005; 131: 417–430. https://doi.org/10.1111/j.1365-2141.2005.05753.x PMID: 16281932

8. Foley JH, Conway EM. Cross Talk Pathways between Coagulation and Inflammation. Circulation Research. Lippincott Williams and Wilkins; 2016. pp. 1392–1408. https://doi.org/10.1161/CIRCRESAHA.116.306853 PMID: 27126649

9. Barnes GD, Kurtz B. Direct oral anticoagulants: Unique properties and practical approaches to management. Heart. BMJ Publishing Group; 2016. pp. 1620–1626. https://doi.org/10.1136/heartjnlp-2015-309075 PMID: 27402803

10. Milesi V, Rebolledo A, Gomez Alvis A, Sanz N, Tommasi J, Drago A, et al. Aspectos estructurales y funcionales de la vena safena humana utilizada como puente aorto-coronario en la cirugía de revascularización miocárdica. Medicina (B Aires). 2001.

11. Gómez-Outes A, Suárez-Gea ML, Lecumberri R, Vargas-Castrillón E. Direct-acting oral anticoagulants: pharmacology, indications, management, and future perspectives. Eur J Haematol. 2015; 95: 389–404. https://doi.org/10.1111/ehj.12610 PMID: 26095540

12. Al Said S, Bode C, Babadagli HE, Basaraba JE, Chen JW, Omar M, et al. The Role of Direct Oral Anticoagulants in Patients With Coronary Artery Disease. J Cardiovasc Pharmacol Ther. 2018; 1074248418795889. https://doi.org/10.1177/1074248418795889 PMID: 30122072

13. Turgeon RD, Ackman ML, Babadagli HE, Basaraba JE, Chen JW, Omar M, et al. The Role of Direct Oral Anticoagulants in Patients With Coronary Artery Disease. J Cardiovasc Pharmacol Ther. 2018; 1074248418795889. https://doi.org/10.1177/1074248418795889 PMID: 30122072

14. Hara T, Fukuda D, Tanaka K, Hayashi Y, Nishimoto S, et al. Rivaroxaban, a novel oral anti-coagulant, attenuates atherosclerotic plaque progression and destabilization in ApoE-deficient mice. Atherosclerosis. 2015; 242: 639–646. https://doi.org/10.1016/j.atherosclerosis.2015.03.023 PMID: 25817329

15. Terry CM, He Y, Cheung AK. Rivaroxaban improves patency and decreases inflammation in a mouse model of catheter thrombosis. Thromb Res. 2016; 144: 106–112. https://doi.org/10.1016/j.thromres.2016.06.008 PMID: 27318247

16. Goto M, Sichiro Miura, Suematsu Y, Idenoto Y, Takata K, Imaizumi S, et al. Rivaroxaban, a factor Xa inhibitor, induces the secondary prevention of cardiovascular events after myocardial ischemia reperfusion injury in mice. Int J Cardiol. 2016; 220: 602–607. https://doi.org/10.1016/j.ijcard.2016.06.212 PMID: 27390997

17. Wu T-C, Chan J-S, Lee C-Y, Leu H-B, Huang P-H, Chen J-S, et al. Rivaroxaban, a factor Xa inhibitor, improves neovascularization in the ischemic hindlimb of streptozotocin-induced diabetic mice. Cardiovasc Diabetol. 2015; 14: 81. https://doi.org/10.1186/s12933-015-0243-1 PMID: 26077117

18. Akkaya G, Bilici Ç, Gençpinar T, Akokay P, Uğurlu B. Effects of rivaroxaban on intimal hyperplasia and smooth muscle cell proliferation at the carotid artery anastomosis site in rabbits. Anatol J Cardiol. 2017; 18: 261–265. https://doi.org/10.14744/AnatolJCardiol.2017.7896 PMID: 29076814

19. Ellinghaus P, Perzborn E, Hauenschild P, Gerdes C, Heitmeier S, Visser M, et al. Expression of pro-inflammatory genes in human endothelial cells: Comparison of rivaroxaban and dabigatran. Thromb Res. 2016; 142: 44–51. https://doi.org/10.1016/j.thromres.2016.04.006 PMID: 2731284

20. Mońko G, Zamaroño-León JJ, Marqués P, Sopeña B, García-García JM, Laich de Koller G, et al. FXa inhibition by rivaroxaban modifies mechanisms associated with the pathogenesis of human abdominal aortic aneurysms. Br J Clin Pharmacol. 2017; 83: 2661–2670. https://doi.org/10.1111/bcp.13383 PMID: 28735510

21. Kondo H, Abe I, Fukui A, Saito S, Miyoshi M, Aoki K, et al. Possible role of rivaroxaban in attenuating pressure-overload-induced atrial fibrosis and fibrillation. J Cardiol. 2018; 71: 310–319. https://doi.org/10.1016/j.jcc.2017.08.007 PMID: 28993090
22. Bode MF, Auriemma AC, Grover SP, Hisada Y, Rennie A, Bode WD, et al. The factor Xa inhibitor rivaroxaban reduces cardiac dysfunction in a mouse model of myocardial infarction. Thromb Res. 2018; 167: 128–134. https://doi.org/10.1016/j.thromres.2018.05.015 PMID: 29843086

23. Liu J, Nishida M, Inui H, Chang J, Zhu Y, Kanno K, et al. Rivaroxaban Suppresses the Progression of Ischemic Cardiomyopathy in a Murine Model of Diet-Induced Myocardial Infarction. J Atheroscler Thromb. 2019; 26: 915–930. https://doi.org/10.5551/jat.48405 PMID: 30687376

24. Ichikawa H, Shimada M, Narita M, Narita I, Kimura Y, Tanaka M, et al. Rivaroxaban, a Direct Factor Xa Inhibitor, Ameliorates Hypertensive Renal Damage Through Inhibition of the Inflammatory Response Mediated by Protease-Activated Receptor Pathway. J Am Heart Assoc. 2019; 8: e012195. https://doi.org/10.1161/JAHA.119.012195 PMID: 30957622

25. Hara T, Fukuda D, Tanaka K, Higashikuni Y, Hirata Y, Yagi S, et al. Inhibition of activated factor X by rivaroxaban attenuates neointima formation after wire-mediated vascular injury. Eur J Pharmaco. 2018; 820: 222–228. https://doi.org/10.1016/j.ejphar.2017.12.037 PMID: 29269019

26. Dittmeier M, Kraft P, Schuhmann MK, Fluri F, Kleinschnitz C. Pretreatment with rivaroxaban attenuates thrombotic protection effects in a model of disseminated intravascular coagulation (DIC) in rats. Blood. 2007. https://doi.org/10.1182/blood.v110.11.935.935

27. Ichikawa H, Shimada M, Narita M, Narita I, Kimura Y, Tanaka M, et al. Rivaroxaban, a Direct Factor Xa Inhibitor, Ameliorates Hypertensive Renal Damage Through Inhibition of the Inflammatory Response Mediated by Protease-Activated Receptor Pathway. J Am Heart Assoc. 2019; 8: e012195. https://doi.org/10.1161/JAHA.119.012195 PMID: 30957622

28. Dittmeier M, Kraft P, Schuhmann MK, Fluri F, Kleinschnitz C. Pretreatment with rivaroxaban attenuates stroke severity in rats by a dual antithrombotic and anti-inflammatory mechanism. Thromb Haemost. 2016; 115: 835–843. https://doi.org/10.1160/TH15-08-0631 PMID: 26489881

29. Rosenkranz AC, Schrör K, Rauch BH. Direct inhibitors of thrombin and factor Xa attenuate clot-induced mitogenesis and inflammatory gene expression in human vascular smooth muscle cells. Thromb Haemost. 2011; 106: 561–2. https://doi.org/10.1160/TH11-04-0275 PMID: 21800011

30. Chen L, Deng H, Cui H, Fang J, Zuo D, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. Impact Journals LLC; 2019. pp. 7204–7218. https://doi.org/10.18632/oncotarget.23208 PMID: 29467962

31. Villari A, Giurdanella G, Bucolo C, Drago F, Salomone S. Apixaban enhances vasodilatation mediated by protease-activated receptor 2 in isolated rat arteries. Front Pharmacol. 2017.8. https://doi.org/10.3389/fphar.2017.00008 PMID: 28154535

32. Da Silva-Santos JE, Chiao CW, Leite R, Webb RC. The Rho-A/Rho-kinase pathway is up-regulated but remains inhibited by cyclic guanosine monophosphate-dependent mechanisms during endotoxemia in small mesenteric arteries. Crit Care Med. 2009. https://doi.org/10.1097/CCM.0b013e31819efb43 PMID: 19325475

33. Perzborn E, Strassburger J, Wilmen A, Pohlmann J, Roehrig S, Schlemmer KH, et al. In vitro and in vivo studies of the novel antithrombotic agent BAY 59–7939—An oral, direct Factor Xa inhibitor. J Thromb Haemost. 2005. https://doi.org/10.1111/j.1538-7836.2005.01166.x PMID: 15748242

34. Fujiwara Y, Ando H, Ushijima K, Horiguchi M, Yamashita C, Fujimura A. Dosing-time-dependent effect of rivaroxaban on coagulation activity in rats. J Pharmaco. 2017. https://doi.org/10.3389/fphar.2017.00008 PMID: 28154535

35. Blessing E, Zhou Q, Bea F, Preusch M, Wang H, Isermann B, et al. Evaluation of plaque stability of advanced atherosclerotic lesions in Apo E-deficient mice after treatment with the oral factor Xa inhibitor rivaroxaban. Mediators Inflamm. 2011;2011. https://doi.org/10.1155/2011/432690 PMID: 21772662

36. Perzborn E, Hirth-Dietrich C, Fischer E, Groth M, Hartmann E, Sperlich-Wull K. Rivaroxaban Has Protective Effects in a Model of Disseminated Intravascular Coagulation (DIC) in Rats. Blood. 2007. https://doi.org/10.1182/blood.v110.11.935.935

37. Weinz C, Buethorn U, Daehler HP, Kohlsdorfer C, Pleiss U, Sandmann S, et al. Pharmacokinetics of BAY 59–7939—An oral, direct Factor Xa inhibitor—In rats and dogs. Xenobiotica. 2005. https://doi.org/10.1080/00498250500260493 PMID: 16308283

38. Kim M, Son H, Noh K, Kim E, Shin B, Kang W. Effects of verapamil and diltiazem on the pharmacokinetics and pharmacodynamics of rivaroxaban. Pharmacuetics11030 133 PMID: 30893910

39. Bonacina F, Moregola A, Porte R, Baragetti A, Bonavita E, Salatin A, et al. Pentraxin 3 deficiency protects from the metabolic inflammation associated to diet-induced obesity. Cardiovasc Res. 2019. https://doi.org/10.1093/cvr/cvz068 PMID: 3089179

40. Bonacina F, Barbieri SS, Cutuli L, Amadio P, Doni A, Sironi M, et al. Vascular pentraxin 3 controls arterial thrombosis by targeting collagen and fibrinogen induced platelets aggregation. Biochim Biophys Acta—Mol Basis Dis. 2016. https://doi.org/10.1016/j.bbadis.2016.03.007 PMID: 26976330
41. Pulakazhi Venu VK, Uboldi P, Dhyani A, Patrini A, Baetta R, Ferri N, et al. Fibronectin extra domain A stabilises atherosclerotic plaques in apolipoprotein E and in LDL-receptor-deficient mice. Thromb Haemost. 2015. https://doi.org/10.1161/TH14-09-0790 PMID: 25881051

42. Bonacina F, Coe D, Wang G, Longhi MP, Baragetti A, Moregola A, et al. Myeloid apolipoprotein E controls dendritic cell antigen presentation and T cell activation. Nat Commun. 2018. https://doi.org/10.1038/s41467-018-05322-1 PMID: 30082777

43. Senden NH, Jeunhomme TM, Heemskerk JW, Wagenvoord R, van’t Veer C, Hemker HC, et al. Factor Xa induces cytokine production and expression of adhesion molecules by human umbilical vein endothelial cells. J Immunol. 1998. PMID: 9780208

44. Papapetropoulos A, Piccardoni P, Cirino G, Bucci M, Sorrentino R, Cicala C, et al. Hypotension and inflammatory cytokine gene expression triggered by factor Xa-nitric oxide signaling. Proc Natl Acad Sci USA. 1998; 95: 4738–42. https://doi.org/10.1073/pnas.95.8.4738 PMID: 959808

45. Schöchl H, van Griensven M, Heitmeier S, Laux V, Kipman U, Roodt J, et al. Dual inhibition of thrombin and activated factor X attenuates disseminated intravascular coagulation and protects organ function in a baboon model of severe Gram-negative sepsis. Crit Care. 2017. https://doi.org/10.1186/s13054-017-1636-y PMID: 28288667

46. Fiusa MML, Carvalho-Filho MA, Annichino-Bizzacchi JM, De Paula E V. Causes and consequences of coagulation activation in sepsis: An evolutionary medicine perspective. BMC Med. 2015. https://doi.org/10.1186/s12916-015-0327-2 PMID: 25943883

47. Hara T, Phuong PT, Fukuda D, Yamaguchi K, Murata C, Nishimoto S, et al. Protease-activated receptor-2 plays a critical role in vascular inflammation and atherosclerosis in apolipoprotein E-deficient mice. Circulation. 2018; 138: 1706–1719. https://doi.org/10.1161/CIRCULATIONAHA.118.033544 PMID: 29700120

48. Jesmin S, Gando S, Zaedi S, Sakuraya F. Differential expression, time course and distribution of four PARs in rats with endotoxin-induced acute lung injury. Inflammation. 2007. https://doi.org/10.1007/s10753-006-9017-8 PMID: 17136598

49. Shih CC, Liao MH, Hsiao TS, Hii HP, Shen CH, Chen SJ, et al. Procaainamide inhibits DNA methylation and alleviates multiple organ dysfunction in rats with endotoxic shock. PLoS One. 2016. https://doi.org/10.1371/journal.pone.0163690 PMID: 27661616

50. Shukla P, Rao GM, Pandey G, Sharma S, Mittapelly N, Shegokar R, et al. Therapeutic interventions in sepsis: current and anticipated pharmacological agents. Br J Pharmacol. 2014; 171: 5011–31. https://doi.org/10.1111/bph.12829 PMID: 24977655

51. Lou X, Yu Z, Yang X, Chen J. Protective effect of rivaroxaban on arteriosclerosis obliterans in rats through modulation of the toll-like receptor 4/NF-κB signaling pathway. Exp Ther Med. 2019. PMID: 31410117

52. Burris RL, Ng H-P, Nagarajan S, Soy protein inhibits inflammation-induced VCAM-1 and inflammatory cytokine induction by inhibiting the NF-κB and AKT signaling pathway in apolipoprotein E-deficient mice. Eur J Nutr. 2014; 53: 135–48. https://doi.org/10.1007/s00394-013-0509-7 PMID: 25468309

53. J.E. DS-S, C.-W. C, R. L, R.C. W. The Rho-A/Rho-kinase pathway is up-regulated but remains inhibited by cyclic guanosine monophosphate-dependent mechanisms during endotoxemia in small mesenteric arteries. Critical Care Medicine. 2009. https://doi.org/10.1097/CCM.0b013e31819e6be4 PMID: 19325475

54. Fink MP. Animal models of sepsis and its complications. Kidney International. 2008. https://doi.org/10.1038/ki.2008.442 PMID: 1887299

55. Shukla P, Rao GM, Pandey G, Sharma S, Mittapelly N, Shegokar R, et al. Therapeutic interventions in sepsis: current and anticipated pharmacological agents. Br J Pharmacol. 2014; 171: 5011–31. https://doi.org/10.1111/bph.12829 PMID: 24977655

56. Shih CC, Liao MH, Hsiao TS, Hii HP, Shen CH, Chen SJ, et al. Procaainamide inhibits DNA methylation and alleviates multiple organ dysfunction in rats with endotoxic shock. PLoS One. 2016. https://doi.org/10.1371/journal.pone.0163690 PMID: 27661616

57. Chen CL, Chen JT, Liang CM, Tai MC, Lu DW, Chen YH. Silibinin treatment prevents endotoxin-induced uveitis in rats in vivo and in vitro. PLoS One. 2017. https://doi.org/10.1371/journal.pone.0174971 PMID: 28376126

58. Levi M, van der Poll T. Coagulation and sepsis. Thrombosis Research. Elsevier Ltd; 2017. pp. 38–44. https://doi.org/10.1016/j.thromres.2016.11.007 PMID: 27886531

59. Jones DP, Patel J. Therapeutic approaches targeting inflammation in cardiovascular disorders. Biology. MDPI AG; 2018. https://doi.org/10.3390/biology7040049 PMID: 3043474

60. Shantsila E, Lip GY. Factor Xa Inhibitors. Non-Vitamin K Antagonist Oral Anticoagulants. Cham: Springer International Publishing; 2016. pp. 25–71. https://doi.org/10.1007/978-3-319-25460-9_3

PLOS ONE | https://doi.org/10.1371/journal.pone.0240669 December 10, 2020 14 / 16
Effects of rivaroxaban on LPS induced vascular inflammation

61. Sanmartín M, Bellmunt S, Cosín-Sales J, García-Moll X, Riera-Mestre A, Almendro-Delia M, et al. Role of rivaroxaban in the prevention of atherosclerotic events. Expert Rev Clin Pharmacol. 2019; 12: 771–780. https://doi.org/10.1080/17512333.2019.1637732 PMID: 31269825

62. Ishibashi Y, Matsu T, Fukami K, Ueda S, Okuda S, Yamagishi S. Rivaroxaban inhibits oxidative and inflammatory reactions in advanced glycation end product-exposed tubular cells by blocking thrombin/protease-activated receptor-2 system. Thromb Res. 2015; 135: 770–7. https://doi.org/10.1016/j.thromres.2015.01.023 PMID: 25634641

63. Posthuma JJ, Posma JJN, van Oerle R, Leenders P, van Gorp RH, Jaminon AMG, et al. Targeting Coagulation Factor Xa Promotes Regression of Advanced Atherosclerosis in Apolipoprotein-E Deficient Mice. Sci Rep. 2019; 9: 130. https://doi.org/10.1038/s41598-018-36956-2 PMID: 30626887

64. Sparkenbaugh EM, Chantranthammachart P, Mickelson J, Van Ryn J, Hebbel RP, Monroe DM, et al. Differential contribution of FXa and thrombin to vascular inflammation in a mouse model of sickle cell disease. Blood. 2014; 123: 1747–1756. https://doi.org/10.1182/blood-2013-08-523936 PMID: 24449213

65. Torramade-Moix S, Palomo M, Vera M, Jerez D, Moreno-Castaño AB, Zafar MU, et al. Apixaban down-regulates Endothelial Inflammation and Prothrombotic Phenotype in an In Vitro Model of Endothelial Dysfunction in Uremia. Cardiovasc Drugs Ther. 2020. https://doi.org/10.1007/s10557-020-07010-z PMID: 32651897

66. Öztürk OH, Çetin A, Özdem SS, Uysal N, Kayişli ÜA, Şentürk ÜK, et al. Plasma levels of nitrites, PGF1α and nitrotyrosine in LPS-treated rats: Functional and histochemical implications in aorta. J Physiol Biochem. 2006; 62: 27–34. https://doi.org/10.1007/BF03165803 PMID: 16909929

67. Bermejo A, Zarzuelo A, Duarte J. In vivo vascular effects of genistein on a rat model of septic shock induced by lipopolysaccharide. J Cardiovasc Pharmacol. 2003; 42: 329–38. https://doi.org/10.1097/00005344-200309000-00003 PMID: 12960677

68. Liao MH, Shih CC, Tsao CM, Chen SJ, Wu CC. RhoA/Rho-Kinase and Nitric Oxide in Vascular Reactivity in Rats with Endotoxemia. PLoS One. 2013. https://doi.org/10.1371/journal.pone.0056331 PMID: 23457552

69. Akahane K, Okamoto K, Kikuchi M, Todoroki H, Higure A, Ohuchi da T, et al. Inhibition of factor Xa suppresses the expression of tissue factor in human monocytes and lipopolysaccharide-induced endothoxia in rats. Surgery. 2001; 130: 809–18. https://doi.org/10.1067/mas.2001.116452 PMID: 11685199

70. Saiededine M, Al-ani B, Cheng CH, Wang L, Hollenberg MD. Rat protease-activated receptor-2 (PAR-2): cDNA sequence and activity of receptor-derived peptides in gastric and vascular tissue. Br J Pharmacol. 1996; 116: 521–530. https://doi.org/10.1111/j.1476-5381.1996.tb15433.x PMID: 8762073

71. Damiano BP, Cheung WM, Santulli RJ, Fung-Leung WP, Ngo K, Ye RD, et al. Cardiovascular responses mediated by protease-activated receptor-2 (PAR-2) and thrombin receptor (PAR-1) are distinguished in mice deficient in PAR-2 or PAR-1. J Pharmacol Exp Ther. 1999; 298: 671–678. PMID: 9919574

72. Kawabata A, Kuroda R, Nakaya Y, Kawai K, Nishikawa H, Kawao N. Factor Xa-evoked relaxation in rat aorta: involvement of PAR-2. Biochem Biophys Res Commun. 2001; 282: 432–5. https://doi.org/10.1006/bbrc.2001.4597 PMID: 11401477

73. Schaeffer P, Mares AM, Dol F, Bono F, Herbert JM. Coagulation factor Xa induces endothelium-dependent relaxations in rat aorta. Circ Res. 1997. https://doi.org/10.1161/01.res.81.5.824 PMID: 9351456

74. Mabley J, Patel JP, Sayer A, Arya R, Scutt G. Direct oral anticoagulant (DOAC)-mediated vasodilation: Role of nitric oxide. Thrombosis Research. 2019. https://doi.org/10.1016/j.thromres.2019.02.014 PMID: 30772641

75. Pham PT, Fukuda D, Yagi S, Kusunose K, Yamada H, Soeki T, et al. Rivaroxaban, a specific FXa inhibitor, improved endothelium-dependent relaxation of aortic segments in diabetic mice. Sci Rep. 2019. https://doi.org/10.1038/s41598-019-47474-0 PMID: 31371788

76. Oe Y, Hayashi S, Fushima T, Sato E, Kisu K, Sato H, et al. Coagulation Factor Xa and Protease-Activated Receptor 2 as Novel Therapeutic Targets for Diabetic Nephropathy. Arterioscler Thromb Vasc Biol. 2016. https://doi.org/10.1161/ATVBAHA.116.307883 PMID: 27283743

77. Guo X, Kolpakov MA, Hooshddaran B, Schappell W, Wang T, Eguchi S, et al. Cardiac Expression of Factor X Mediates Cardiac Hypertrophy and Fibrosis in Pressure Overload. JACC Basic to Transl Sci. 2020. https://doi.org/10.1016/j.jacbts.2019.10.006 PMID: 32043021

78. Nakaniishi N, Kaikita K, Ishi M, Mitsuse T, Oimatsu Y, Tsujita K. Effects of rivaroxaban on cardiac remodeling after experimental myocardial infarction. Circ Conf. 2018.

79. Meng S, Jiechun H, Xiaotian S, Fangrui W, Xianglin C, Rongrong J, et al. Effect of rivaroxaban on the injury during endotoxin-induced damage to human umbilical vein endothelial cells. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue. 2019; 31: 468–473. https://doi.org/10.3760/cma.j.issn.2095-4352.2019.04.019 PMID: 31109423
80. Álvarez E, Paradela-Doborro B, Raposeiras-Roubín S, González-Juanatey JR. Protective, repairing and fibrinolytic effects of rivaroxaban on vascular endothelium. Br J Clin Pharmacol. 2018. https://doi.org/10.1111/bcp.13440 PMID: 28940408