Biochemical and Biophysical Research Communications 460 (2015) 691–696

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Thrombin generation in abdominal sepsis is Rho-kinase-dependent

Yongzhi Wang [a], Oscar Ö. Braun [b], Su Zhang [a], Eva Norström [c], Henrik Thorlacius [a, *]

[a] Department of Clinical Sciences, Malmö, Section for Surgery, Malmö, Sweden
[b] Department of Clinical Sciences, Lund, Section for Cardiology, Lund, Sweden
[c] Department of Translational Medicine, Malmö, Section of Clinical Chemistry, Malmö, Sweden

ARTICLE INFO

Article history:
Received 13 February 2015
Available online 24 March 2015

Keywords:
Coagulation
Inflammation
Sepsis
Thrombin

ABSTRACT

Sepsis causes severe derangements of the coagulation system. However, the signaling mechanisms regulating sepsis-induced thrombin generation remain elusive. Herein, we hypothesized that Rho-kinase might be an important regulator of thrombin generation in abdominal sepsis. Abdominal sepsis was induced by cecal ligation and puncture (CLP) in C57Bl/6 mice. Thrombin generation, coagulation factors, lung histology and myeloperoxidase (MPO) activity were determined 6 h and 24 h after induction of CLP. Induction of CLP triggered a systemic inflammatory response characterized by neutrophil accumulation and tissue injury in the lung as well as thrombocytopenia and leukocytopenia. Administration of Y-27632, a Rho-kinase inhibitor, attenuated these markers of systemic inflammation in CLP animals. Moreover, peak thrombin formation was decreased by 77% and 81% in plasma from mice 6 h and 24 h after induction of CLP. Total thrombin generation was reduced by 64% and 67% 6 h and 24 h after CLP induction, respectively. Notably, administration of Y-27632 increased peak formation by 99% and total thrombin generation by 66% in plasma from septic animals. In addition, CLP markedly decreased plasma levels of prothrombin, factor V and factor X at 6 h and 24 h. Interestingly, Rho-kinase inhibition significantly enhanced levels of prothrombin, factor V and factor X in plasma from septic mice. In addition, inhibition of Rho-kinase decreased CLP-induced elevations of CXCL2 by 36% and interleukin-6 by 38%. These novel findings suggest that sepsis-induced thrombin generation is regulated by Rho-kinase. Moreover, inhibition of Rho-kinase reverses sepsis-evoked consumption of coagulation factors. Thus, our results show that targeting Rho-kinase signaling might protect against coagulation dysfunction in abdominal sepsis.

1. Introduction

Abdominal sepsis is a significant cause of mortality rising up to as high as 35% in the presence of disseminated intravascular coagulation [1–3]. Contamination of the abdominal cavity with bacterial toxins and microbes trigger systemic activation of the immune system via secretion of pro-inflammatory compounds, including CXCL2 (macrophage inflammatory protein-2) and interleukin-6 (IL-6) [4,5]. Microbial products and cytokines promote tissue factor activation leading to disturbances of hemostasis, such as an initial hypercoagulable state with impaired anti-coagulation and fibrinolysis with increased risk of intravascular fibrin clot formation and subsequent tissue ischemia and damage [6–8]. Thrombin is generated by proteolytic cleavage of prothrombin, which is secreted from hepatocytes [9]. Moreover, thrombin is a potent pro-inflammatory mediator activating monocytes and endothelial cells via specific protease-activated receptors [10]. However, the signaling mechanisms underlying thrombin generation in abdominal sepsis remain elusive.

Antibiotics are the only specific treatment available. It is therefore interesting to note that statin intake might reduce mortality and progression of microbial infections in septic patients [11–13]. A protective effect of statins in sepsis is also supported by experimental data showing that treatment with statins protects the lungs and increase survival in sepsis [14,15]. Statins reduce thrombin generation in patients with cardiovascular diseases [16] and are mainly used to regulate hypercholesterolemia by inhibiting HMG-CoA reductase, which is the rate-limiting enzyme in the formation of mevalonate [17]. Mevalonate is also a precursor of farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which

* Corresponding author. Department of Clinical Sciences, Malmö, Section of Surgery, Lund University, Skåne University Hospital, S-205 02 Malmö, Sweden. Fax: +46 40 336207.
E-mail address: henrik.thorlacius@med.lu.se (H. Thorlacius).

http://dx.doi.org/10.1016/j.bbrc.2015.03.091
0006-291X/

are critical in protein isoprenylation of regulatory proteins, such as small G proteins of the Rho family needed for a broad range of functions, such as cell adhesion, migration, cytoskeletal organization, apoptosis, and reactive oxygen species formation [18–21]. Inhibition of Rho-kinase has been shown to protect against endotoxemic liver injury [22] and septic lung damage [23]. Nonetheless, the potential role of Rho-kinase in regulating thrombin generation and coagulation factor consumption in abdominal sepsis is not known.

Thus, the aim of this study was to determine the role of Rho-kinase signaling in sepsis-induced coagulation in vivo. For this purpose, a model based on ligation and puncture of the cecum in mice was used.

2. Materials and methods

2.1. Animals

Animals were handled in accordance with the legislation on the protection of animals and all experiments were approved by the Regional Ethical Committee for Animal Experimentation at Lund University, Sweden (application number M310-12, approval date 12 december 2012). Male C57Bl/6 mice (20–25 g were housed in a with 12-12 h light dark cycle at 22 °C, and fed a laboratory diet and water *ad libitum*. Animals were anesthetized with 75 mg of ketamine hydrochloride (Hoffman–La Roche, Basel, Switzerland) and 25 mg of xylazine (Janssen Pharmaceutica, Beerse, Belgium) per kg.

2.2. Experimental protocol of sepsis

Animals were pretreated with vehicle, i.e. phosphate-buffered saline (PBS, $n = 5$) or 0.5, 2.5 and 10 mg/kg of Y-27632 ($n = 5$) 15 min prior to induction of cecal ligation and puncture (CLP). Y-27632 selectively targets Rho-kinase with exception of protein kinase C-related protein kinase 2 [24,25]. Previous studies have shown that 10 mg/kg of Y-27632 exerts a protective effect in models of abdominal sepsis, cholestasis and colonic ischemia-reperfusion [23,26,27]. CLP was used to induce polymicrobial sepsis as described previously [28]. In brief, mice were anesthetized and a midline incision was made to expose the cecum, which was filled with feces by milking stool backwards from the ascending colon and 75% of the cecum was ligated. The cecum was soaked with PBS and punctured twice (21-gauge needle) on the antimesenteric border. The cecum was returned into the peritoneal cavity and the abdominal incision was closed. Sham mice ($n = 5$) underwent identical procedures, but the cecum was neither ligated nor punctured. Animals were re-anesthetized 6 h and 24 h after CLP for collection of samples for further analysis.

2.3. Histology

Lungs samples were fixed in 4% formaldehyde buffer overnight and then dehydrated and paraffin-embedded. Six μm sections were stained with hematoxylin and eosin. Lung injury was quantified in a blinded manner by use of a modified scoring system as previously described [29], including size of alveolar spaces, thickness of alveolar septa, alveolar hemorrhage and neutrophil infiltration graded on a 0 (absent) to 4 (extensive) scales.

2.4. MPO levels

Lung tissues were snap frozen and lung levels of MPO levels were assayed according to a standard protocol [29]. Briefly, lung tissue was thawed and homogenized in 0.02 M PB (pH 7.4). Supernatant was discarded after centrifugation for 10 min at 14,000 rpm and the pellet was dissolved by adding one mL of 0.5% hexadecyltrimethylammonium bromide. Samples were stored at −20 °C overnight and then thawed and kept at 60 °C water bath for 2 h followed by 90 s sonication. Supernatant was collected after 5 min centrifugation at 14,000 rpm and the MPO activity in the supernatant was determined spectrophotometrically as the MPO-catalyzed change in absorbance in the redox reaction of H_2O_2 (450 nm, with a reference filter 540 nm, 25 °C). Values were expressed as MPO units per g tissue.

2.5. Circulating leukocyte and platelet counts

Tail vein blood was mixed with Turks solution (0.2 mg gentian violet in 1 mL glacial acetic acid, 6.25% v/v) 1:20 for quantification of polymorphonuclear (PMNL) and monomorphonuclear (MNL) cells or with stromatol solution (Mascia Brunelli spa, Viale Monza, Milan, Italy) 1:500 for identification of platelets in a hematocytometer chamber.

2.6. Enzyme-linked immunosorbent assay

Plasma was collected 6 h and 24 h after induction of CLP. Commercially available ELISA kits were used to quantify plasma levels of prothrombin, factor V and factor X (Molecular innovations, Peary Ct, Novi, USA and USCN Life Science, Wuhan, Hubei, PRC) as well as CXCL2 and IL-6 (R & D Systems, Abingdon, Oxon, UK). Linearity was assessed and confirmed by samples containing high concentrations of recombinant mouse prothrombin, factor V, factor X, CXCL2 and IL-6 serially diluted with a calibrator diluent.

2.7. Thrombin generation

Mouse blood was collected from the inferior vena cava with 1:10 acid citrate dextrose. As previously described [5], platelet rich plasma (PRP) was prepared immediately after blood sampling by centrifugation at 200 × g (3 min). Platelet poor plasma (PPP) was obtained by a series of centrifugations to remove platelets and cell fragments: samples were first centrifuged at 2500 × g (15 min), and then at 10,000 × g (5 min) and finally samples were centrifuged at 19,000 × *g* (4 min) to obtain PPP. Recombinant mouse tissue factor (1 pM, R&D Systems, Abingdon, Oxon, UK) was prepared in Hepes-buffered saline containing 20 mM Hepes, 140 mM NaCl and 5 mg/mL bovine serum albumin (BSA) and a mixture of fluorogenic substrate, Z-Gly-Gly-Arg-AMC, (2.5 mM, Bachem, Bubendorf, Switzerland) and $CaCl_2$ (16.6 mM) was freshly prepared in buffer containing 20 mM Hepes and 60 mg/mL BSA (pH 7.35). To initiate thrombin generation, 10 μL PRP, 30 μL PPP, 10 μL tissue factor and 10 μL fluorogenic substrate with $CaCl_2$ were mixed. The fluorescence intensity was measured using a SpectraMax Gemini fluorometer (Sunnyvale, CA, USA) at an excitation wavelength of 390 nm and an emission wavelength of 460 nm. All experiments were carried out in duplicates and all data presented are individual biological replicates.

2.8. Statistics

Data are presented as mean values ± standard error of the mean (SEM). Statistical evaluations were performed using the non-parametric Mann–Whitney test for comparisons between two groups. $P < 0.05$ was considered statistically significant and n represents the number of animals.

3. Results

3.1. Lung injury and neutrophil infiltration in sepsis

Histological examination showed normal lung structure in sham mice (Fig. 1A). CLP provoked destruction of the lung tissue micro-architecture typified by edema formation, capillary congestion and neutrophil infiltration (Fig. 1B). Quantification of the morphological changes showed that the lung damage score was increased in CLP mice (Fig. 1D). Administration of the Rho-kinase inhibitor Y-27632 protected against sepsis-induced pulmonary damage and decreased the lung injury score by 67% (Fig. 1D). Herein, it was found that CLP induction significantly enhanced MPO activity in the lung (Fig. 1E). CLP decreased the number of circulating leukocytes and platelets (Table 1). Y-27632 decreased pulmonary activity of MPO (Fig. 1E) as well as reversed the sepsis-induced reduction in circulating numbers of platelets and leukocytes (Table 1).

Table 1
Systemic platelet and leukocyte differential counts.

	Platelets	MNL	PMNL	Total leukocytes
Sham 6h	1186 ± 22	6.3 ± 0.4	1.9 ± 0.3	8.2 ± 0.3
Sham 24h	1187 ± 40	6.2 ± 0.3	1.8 ± 0.4	7.9 ± 0.3
CLP 6h + PBS	1057 ± 47	5.9 ± 0.3	1.0 ± 0.2[a]	6.9 ± 0.1[a]
CLP 24h + PBS	651 ± 33[a]	1.3 ± 0.2[a]	0.4 ± 0.2[a]	1.7 ± 0.2[a]
CLP 6h + Y-27632	1110 ± 21[b]	5.6 ± 0.3[b]	3.2 ± 0.4[a,b]	8.8 ± 0.4[b]
CLP 24h + Y-27632	1084 ± 41[b]	6.0 ± 0.3[b]	2.3 ± 0.3[a,b]	8.3 ± 0.4[b]

Blood was collected from sham animals receiving PBS as well as from mice pre-treated i.p. with vehicle (PBS) or Y-27632 (10 mg/kg) 15 min prior to CLP challenge. Leukocytes were identified as monomorphonuclear leukocytes (MNL) and polymorphonuclear leukocytes (PMNL). Data represents mean ± SEM, 10^6 cells/mL and $n = 5$. [a]$P < 0.05$ *vs.* sham, [b]$P < 0.05$ *vs.* CLP + PBS.

3.2. Rho-kinase regulates sepsis-induced thrombin generation

Representative time-dependent curves of thrombin generation are depicted in Fig. 2A showing that thrombin generation was markedly decreased in plasma harvested from animals 6 h and 24 h after CLP induction. For example, peak formation of thrombin was reduced by 81% (Fig. 2C) and total thrombin generation was decreased by 67% in plasma from mice exposed to CLP for 24 h (Fig. 2D). Time to peak formation of thrombin was only increased 6 h after CLP (Fig. 2B). Administration of Y-27632 dose-dependently restored thrombin generation in plasma from septic animals (Fig. 2). For example, injection of 10 mg/kg of Y-27632 increased

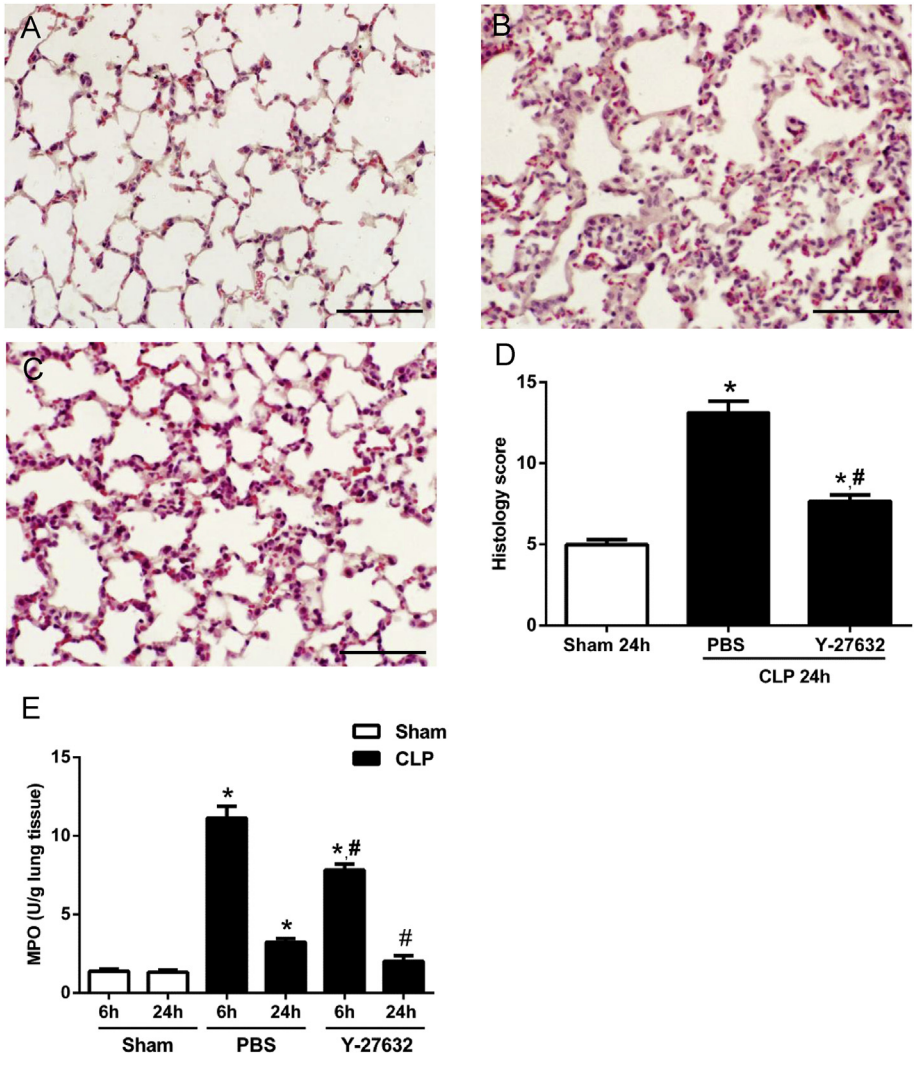

Fig. 1. Systemic inflammation in the CLP model. Representative hematoxylin & eosin sections of lung tissue from A) sham animals and from mice 24 h after CLP induction pretreated with B) vehicle or C) the Rho-kinase inhibitor Y-27632 (10 mg/kg). Scale bar indicates 100 μm. D) Lung injury score was calculated as described in Materials and methods. E) Lung activity of MPO (U/g tissue) quantified 6 h after CLP induction. Data represent mean ± SEM and $n = 5$. *$P < 0.05$ vs. Sham and #$P < 0.05$ vs. PBS + CLP.

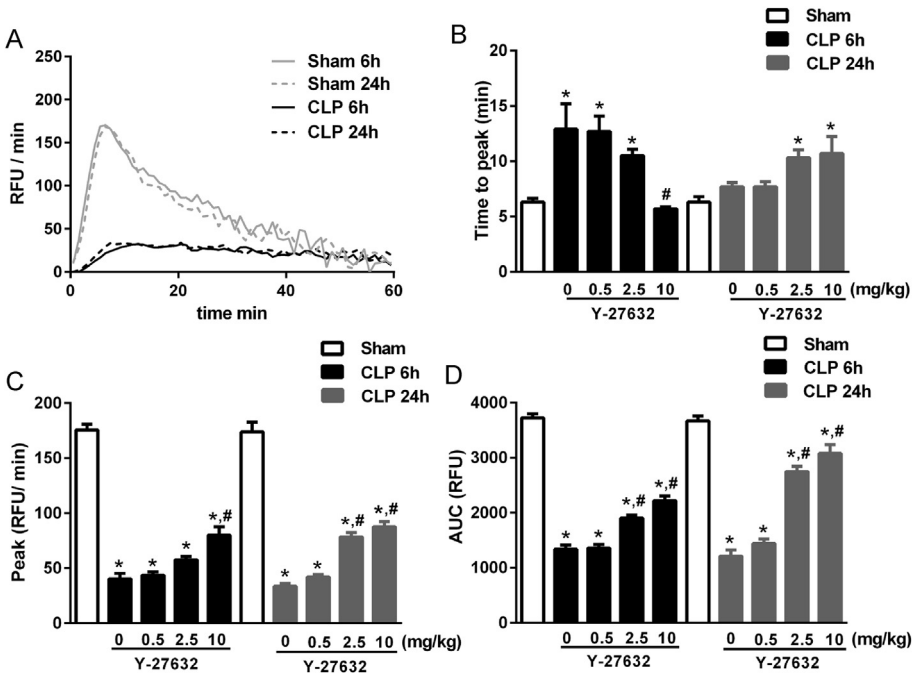

Fig. 2. CLP-induced thrombin generation. Plasma samples were harvested 6 h and 24 h after CLP induction. Animals were pretreated with the Rho-kinase inhibitor Y-27632 (0.5-10 mg/kg) or vehicle. Sham-operated animals served as negative controls. A) thrombin generation over time, B) time to peak formation, C) peak levels and D) total thrombin generation were determined as described in Materials and methods. Data represent mean ± SEM and $n = 5$. $^{*}P < 0.05$ *vs.* Sham and $^{\#}P < 0.05$ vs. PBS + CLP.

total thrombin generation by 66% and 151% at 6 h and 24 h, respectively, after CLP (Fig. 2D).

3.3. *Rho-kinase regulates consumption of coagulation factors in sepsis*

CLP decreased plasma levels of prothrombin by 73% at 6 h and by 65% at 24 h (Fig. 3A). In addition, CLP depressed plasma levels of factor V by 48% at 6h (Fig. 3B). Plasma levels of factor V were not significantly reduced 24 h after CLP induction (Fig. 3B). CLP reduced plasma levels of factor X at 6 h and 24 h by 64% and 63%, respectively (Fig. 3C). Administration of Y-27632 increased plasma levels of prothrombin in CLP animals. For example, Y-27632 enhanced prothrombin levels in plasma by 37% (Fig. 3A). Moreover, inhibition of Rho-kinase increased levels of factor V at 6 h and factor X at both 6 h and 24 h after induction of CLP (Fig. 3B and C).

3.4. *Rho-kinase regulates secretion of pro-inflammatory compounds in sepsis*

No detectable level of CXCL2 was observed in sham animals (Fig. 3D). CLP markedly increased plasma levels of CXCL2 (Fig. 3D) at 6 h and 24 h. Administration of Y-27632 decreased CLP-induced plasma levels of CXCL2 by 42% at 6 h and by 36% at 24 h (Fig. 3D). Moreover, plasma IL-6 levels were greatly enhanced 6 h and 24 h after induction of CLP (Fig. 3E). Inhibition of Rho-kinase decreased plasma levels of IL-6 by 38% and 43% 6 h and 24 h after CLP induction, respectively (Fig. 3E).

4. Discussion

Sepsis is associated with systemic derangement of the coagulation system, however, the signaling pathways regulating sepsis-induced coagulopathy remain elusive. Herein, we demonstrate that the Rho-kinase inhibitor Y-27632 restored thrombin generation in septic animals. Moreover, Rho-kinase inhibition reversed sepsis-evoked depletion of major coagulation factors. Thus, these findings suggest that Rho-kinase regulates significant alterations of hemostasis observed in abdominal sepsis.

Systemic inflammatory response syndrome triggers complex changes in the coagulation system. The current concept is that patients with sepsis undergo dynamic changes typified by an early hypercoagulable state followed by a subsequent hypocoagulable phase [30]. The knowledge about the coagulant response to severe infections has been limited by traditional methods to monitor coagulation status by quantification of individual elements in the coagulation cascade, such as coagulation factors and inhibitors, which may not capture the global effect on hemostasis. More recently, global hemostasis assays, such as the thrombin generation test and thromboelastometry have been developed to obtain a more comprehensive and biologically relevant evaluation of hemostasis. Multiple studies have shown the usefulness of thrombin generation in various conditions, including chronic liver disease [31], trauma-induced coagulopathy [32] and sepsis [5]. However, up to date, the knowledge about regulatory signaling pathways in sepsis-induced thrombin generation is limited. Herein, we found that inhibition of Rho-kinase significantly reversed the sepsis-induced depression of thrombin generation. This is the first study showing that Rho-kinase activity regulates thrombin generation in vivo. In this context, it is interesting to note that platelets have been reported to control thrombin generation in sepsis [33] and that Rho proteins are known to regulate numerous features of platelet physiology, including aggregation, granule secretion, filopodia formation and clot retraction [34]. Thus, it is possible that Rho-kinase in platelets is the target of Y-27632, which will be addressed in future studies. Nonetheless, considering the potent pro-inflammatory and pro-coagulative effects of thrombin [35], this inhibitory effect of Y-27632 might help explain the protective effect of targeting Rho-kinase signaling in abdominal sepsis [23]. Notably, recent studies have reported that statins, such simvastatin, reduce thrombin generation in different conditions, such as acute ST-segment elevation myocardial infarction [36] and

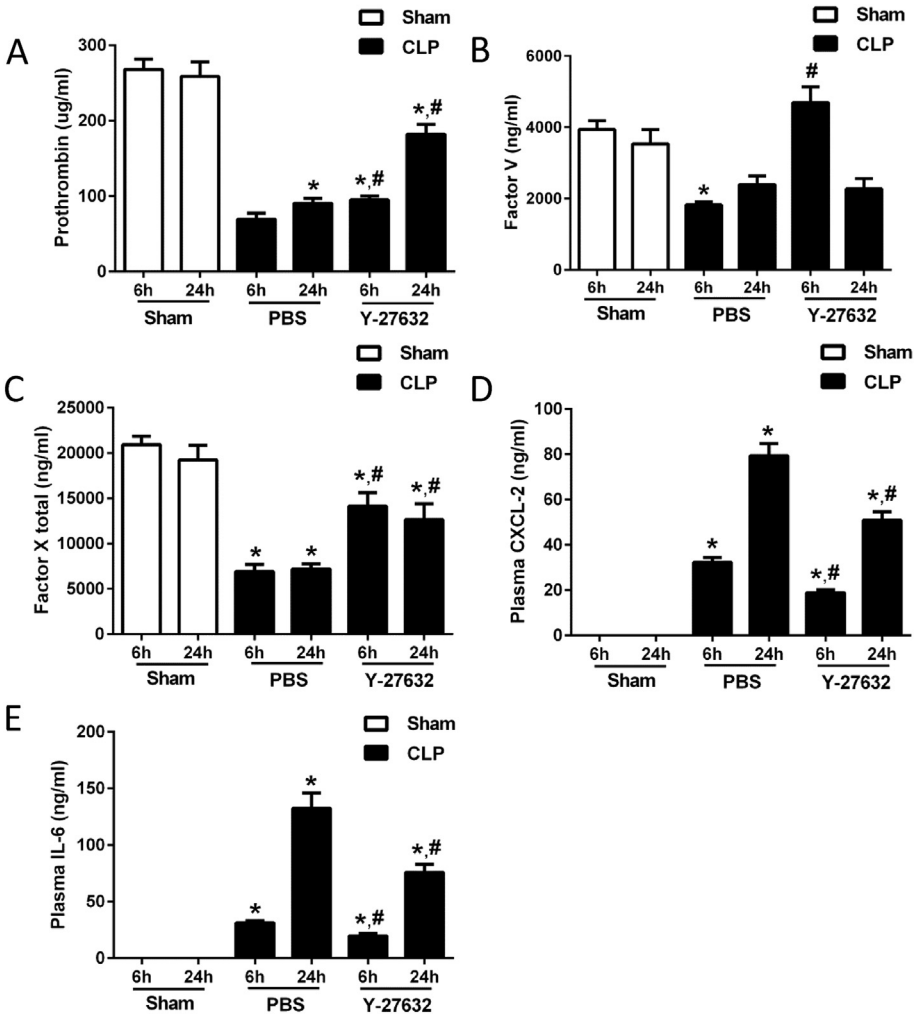

Fig. 3. CLP-induced depletion of coagulation factors. Plasma samples were harvested 6 h and 24 h after CLP induction. Animals were pretreated with the Rho-kinase inhibitor Y-27632 (10 mg/kg) or vehicle. Sham-operated animals served as negative controls. Plasma levels of A) prothrombin, B) factor V, C) factor X, D) CXCL2 and E) IL-6 were determined by use of ELISA. Data represent mean ± SEM and $n = 5$. $^{*}P < 0.05$ *vs.* Sham and $^{\#}P < 0.05$ vs. PBS + CLP.

hypercholesterolemia [37]. Considering that statins prevent isoprenylation of small guanosine triphosphatases, such as Rac1 and Rho, which are necessary for their function [38], our current results showing that Rho-kinase plays an important role in regulating thrombin generation might help explain the beneficial impact of statins on the coagulation system.

Secretion of pro-inflammatory cytokines is considered to drive inflammation-induced activation of the coagulation system. Cytokines stimulates tissue factor expression on monocytes, which binds to activated factor VII and this complex catalyzes the formation of activated factor X. Factor V, prothrombin, calcium and activated factor X constitute the prothrombinase complex, which is the rate-limiting step in thrombin generation [39]. For example, IL-6 is a pro-inflammatory cytokine that can induce a prothrombotic state by increasing platelet production and reactivity as well as promoting expression of tissue factor and coagulation factors [39]. Indeed, one recent study has demonstrated that IL-6 can increase thrombin generation [39]. In the present study, it was found that CLP triggered increased plasma levels of IL-6 and that administration of Y-27632 abolished the sepsis-induced increase in IL-6 production. Moreover, we observed that CLP markedly decreased plasma levels of prothrombin, factor V and factor X, suggesting a substantial consumption of these coagulation factors in abdominal sepsis. A similar decrease in plasma levels of prothrombin, factor V and factor X has been observed in patients with sepsis [40]. Interestingly, administration of Y-27632 reversed the sepsis-induced reduction in prothrombin, factor X at 6h and 24h as well as factor V at 6h, indicating that Rho-kinase signaling is important for inflammation-driven activation and consumption of coagulation factors in abdominal sepsis. As shown before [23], we found that Y-27632 inhibited sepsis-evoked neutrophil recruitment and lung damage as well as thrombocytopenia and leukocytopenia, supporting a broad and potent anti-inflammatory impact exerted by Y-27632 in abdominal sepsis. We found that Y-27632 significantly decreased CXCL2 formation, which is a powerful stimulator of neutrophils. This inhibitory effect of Y-27632 on CXCL2 formation might help explain the inhibitory effect of Y-27632 on neutrophil accumulation in the lung as suggested previously [23]. In this context, considering the important role of Rho-kinase in both thrombin generation and neutrophil recruitment it could be suggested that Rho-kinase might be a key link in the crosstalk between inflammation and coagulation in sepsis. Future studies will be performed to identify potential immune cells involved in the Rho-kinase-dependent regulation of sepsis-driven coagulation.

Taken together, these results suggest that Rho-kinase signaling is an important component in sepsis-associated thrombin generation and coagulation factor consumption. These potent anti-coagulative effects of targeting Rho-kinase activity not only help

explaining the central role of Rho-kinase signaling in abdominal sepsis but also suggest that inhibition of Rho-kinase might be a useful way to improve the coagulative status in patients with sepsis.

Conflict of interest

None.

Acknowledgments

This work was supported by grants from the Swedish Medical Research Council (2012-3685), Crafoordska stiftelsen, Einar och Inga Nilssons stiftelse, Harald och Greta Jaenssons stiftelse, Greta och Johan Kocks stiftelser, Fröken Agnes Nilssons stiftelse, Franke och Margareta Bergqvists stiftelse för främjande av cancer-forskning, Magnus Bergvalls stiftelse, Mossfelts stiftelse, Nanna Svartz stiftelse, Ruth och Richard Julins stiftelse, Svenska Läkaresällskapet, Allmäna sjukhusets i Malmö stiftelse för bekämpande av cancer, SUS fonder, Skåne University Hospital and Lund University. Yongzhi Wang is supported by a grant from China.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.03.091.

References

[1] K.M. Kaukonen, M. Bailey, S. Suzuki, D. Pilcher, R. Bellomo, Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012, JAMA 311 (2014) 1308–1316.
[2] H. Saito, I. Maruyama, S. Shimazaki, Y. Yamamoto, N. Aikawa, R. Ohno, A. Hirayama, T. Matsuda, H. Asakura, M. Nakashima, N. Aoki, Efficacy and safety of recombinant human soluble thrombomodulin (ART-123) in disseminated intravascular coagulation: results of a phase III, randomized, double-blind clinical trial, J. Thromb. Haemost. 5 (2007) 31–41.
[3] R. Ferrer, I. Martin-Loeches, G. Phillips, T.M. Osborn, S. Townsend, R.P. Dellinger, A. Artigas, C. Schorr, M.M. Levy, Empiric antibiotic treatment reduces mortality in severe sepsis and septic shock from the first hour: results from a guideline-based performance improvement program, Crit. Care Med. 42 (2014) 1749–1755.
[4] M. Bhatia, R.L. Zemans, S. Jeyaseelan, Role of chemokines in the pathogenesis of acute lung injury, Am. J. Respir. Cell. Mol. Biol. 46 (2012) 566–572.
[5] Y. Wang, O.B. O, S. Zhang, L. Luo, E. Norstrom, H. Thorlacius, Dynamic changes in thrombin generation in abdominal sepsis in mice, Shock 42 (2014) 343–349.
[6] M. Levi, T. van der Poll, The role of natural anticoagulants in the pathogenesis and management of systemic activation of coagulation and inflammation in critically ill patients, Semin. Thromb. Hemost. 34 (2008) 459–468.
[7] A.D. Shaw, G.M. Vail, D.J. Haney, J. Xie, M.D. Williams, Severe protein C deficiency is associated with organ dysfunction in patients with severe sepsis, J. Crit. Care 26 (2011) 539–545.
[8] N. Semeraro, C.T. Ammollo, F. Semeraro, M. Colucci, Sepsis, thrombosis and organ dysfunction, Thromb. Res. 129 (2012) 290–295.
[9] N. Mackman, Role of tissue factor in hemostasis, thrombosis, and vascular development, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 1015–1022.
[10] K. Johnson, Y. Choi, E. DeGroot, I. Samuels, A. Creasey, L. Aarden, Potential mechanisms for a proinflammatory vascular cytokine response to coagulation activation, J. Immunol. 160 (1998) 5130–5135.
[11] H. Yasuda, P.S. Yuen, X. Hu, H. Zhou, R.A. Star, Simvastatin improves sepsis-induced mortality and acute kidney injury via renal vascular effects, Kidney Int. 69 (2006) 1535–1542.
[12] P.S. Kruger, M.L. Harward, M.A. Jones, C.J. Joyce, K.M. Kostner, M.S. Roberts, B. Venkatesh, Continuation of statin therapy in patients with presumed infection: a randomized controlled trial, Am. J. Respir. Crit. Care Med. 183 (2011) 774–781.
[13] A.P. Liappis, V.L. Kan, C.G. Rochester, G.L. Simon, The effect of statins on mortality in patients with bacteremia, Clin. Infect. Dis. 33 (2001) 1352–1357.
[14] S. Zhang, M. Rahman, S. Zhang, Z. Qi, H. Thorlacius, Simvastatin antagonizes CD40L secretion, CXC chemokine formation, and pulmonary infiltration of neutrophils in abdominal sepsis, J. Leukoc. Biol. 89 (2011) 735–742.
[15] M.W. Merx, E.A. Liehn, U. Janssens, R. Lutticken, J. Schrader, P. Hanrath, C. Weber, HMG-CoA reductase inhibitor simvastatin profoundly improves survival in a murine model of sepsis, Circulation 109 (2004) 2560–2565.
[16] F. Violi, C. Calvieri, D. Ferro, P. Pignatelli, Statins as antithrombotic drugs, Circulation 127 (2013) 251–257.
[17] A. Endo, Y. Tsujita, M. Kuroda, K. Tanzawa, Inhibition of cholesterol synthesis in vitro and in vivo by ML-236A and ML-236B, competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, Eur. J. Biochem. 77 (1977) 31–36.
[18] A. Malliri, J.G. Collard, Role of Rho-family proteins in cell adhesion and cancer, Curr. Opin. Cell. Biol. 15 (2003) 583–589.
[19] Y. Wang, J. Roller, J.E. Slotta, S. Zhang, L. Luo, M. Rahman, I. Syk, M.D. Menger, H. Thorlacius, Distinct patterns of leukocyte recruitment in the pulmonary microvasculature in response to local and systemic inflammation, Am. J. Physiol. Lung Cell. Mol. Physiol. 304 (2013) L298–L305.
[20] Y. Zhu, P.J. Casey, A.P. Kumar, S. Pervaiz, Deciphering the signaling networks underlying simvastatin-induced apoptosis in human cancer cells: evidence for non-canonical activation of RhoA and Rac1 GTPases, Cell. Death Dis. 4 (2013) e568.
[21] J. Park, Y. Gu, Y. Lee, Z. Yang, Y. Lee, Phosphatidic acid induces leaf cell death in Arabidopsis by activating the Rho-related small G protein GTPase-mediated pathway of reactive oxygen species generation, Plant Physiol. 134 (2004) 129–136.
[22] J.E. Slotta, M.W. Laschke, M.D. Menger, H. Thorlacius, Rho-kinase signalling mediates endotoxin hypersensitivity after partial hepatectomy, Br. J. Surg. 95 (2008) 976–984.
[23] Z. Hasan, K. Palani, M. Rahman, S. Zhang, I. Syk, B. Jeppsson, H. Thorlacius, Rho-kinase signaling regulates pulmonary infiltration of neutrophils in abdominal sepsis via attenuation of CXC chemokine formation and Mac-1 expression on neutrophils, Shock 37 (2012) 282–288.
[24] T. Ishizaki, M. Uehata, I. Tamechika, J. Keel, K. Nonomura, M. Maekawa, S. Narumiya, Pharmacological properties of Y-27632, a specific inhibitor of rho-associated kinases, Mol. Pharmacol. 57 (2000) 976–983.
[25] S.P. Davies, H. Reddy, M. Caivano, P. Cohen, Specificity and mechanism of action of some commonly used protein kinase inhibitors, Biochem. J. 351 (2000) 95–105.
[26] M.W. Laschke, S. Dold, M.D. Menger, B. Jeppsson, H. Thorlacius, The Rho-kinase inhibitor Y-27632 inhibits cholestasis-induced platelet interactions in the hepatic microcirculation, Microvasc. Res. 78 (2009) 95–99.
[27] S. Santen, Y. Wang, M.W. Laschke, M.D. Menger, B. Jeppsson, H. Thorlacius, Rho-kinase signalling regulates CXC chemokine formation and leukocyte recruitment in colonic ischemia-reperfusion, Int. J. Colorectal Dis. 25 (2010) 1063–1070.
[28] D. Rittirsch, M.S. Huber-Lang, M.A. Flierl, P.A. Ward, Immunodesign of experimental sepsis by cecal ligation and puncture, Nat. Protoc. 4 (2009) 31–36.
[29] L. Luo, S. Zhang, Y. Wang, M. Rahman, I. Syk, E. Zhang, H. Thorlacius, Proinflammatory role of neutrophil extracellular traps in abdominal sepsis, Am. J. Physiol. Lung Cell. Mol. Physiol. 307 (2014) L586–L596.
[30] S.K. Picoli-Quaino, B.E. Alves, V.B. Faiotto, S.A. Montalvao, C.A. De Souza, J.M. Annichino-Bizzacchi, E.V. De Paula, Impairment of thrombin generation in the early phases of the host response of sepsis, J. Crit. Care 29 (2014) 31–36.
[31] A. Tripodi, Q.M. Anstee, K.K. Sogaard, M. Primignani, D.C. Valla, Hypercoagulability in cirrhosis: causes and consequences, J. Thromb. Haemost. 9 (2011) 1713–1723.
[32] J.L. Kashuk, E.E. Moore, M. Wohlauer, J.L. Johnson, M. Pezold, J. Lawrence, W.L. Biffl, C.C. Burlew, C. Barnett, M. Sawyer, A. Sauaia, Initial experiences with point-of-care rapid thrombelastography for management of life-threatening postinjury coagulopathy, Transfusion 52 (2012) 23–33.
[33] T.J. Gould, T.T. Vu, L.L. Swystun, D.J. Dwivedi, S.H. Mai, J.I. Weitz, P.C. Liaw, Neutrophil extracellular traps promote thrombin generation through platelet-dependent and platelet-independent mechanisms, Arterioscler. Thromb. Vasc. Biol. 34 (2014) 1977–1984.
[34] J.E. Aslan, O.J. McCarty, Rho GTPases in platelet function, J. Thromb. Haemost. 11 (2013) 35–46.
[35] C.T. Esmon, K. Fukudome, T. Mather, W. Bode, L.M. Regan, D.J. Stearns-Kurosawa, S. Kurosawa, Inflammation, sepsis, and coagulation, Haematologica 84 (1999) 254–259.
[36] M. Pastuszczak, A. Kotlarz, M. Mostowik, J. Zalewski, K. Zmudka, A. Undas, Prior simvastatin treatment is associated with reduced thrombin generation and platelet activation in patients with acute ST-segment elevation myocardial infarction, Thromb. Res. 125 (2010) 382–386.
[37] A. Szczeklik, J. Musial, A. Undas, P. Gajewski, P. Gora, J. Swadzba, M. Jankowski, Inhibition of thrombin generation by simvastatin and lack of additive effects of aspirin in patients with marked hypercholesterolemia, J. Am. Coll. Cardiol. 33 (1999) 1286–1293.
[38] Y. Rikitake, J.K. Liao, Rho GTPases, statins, and nitric oxide, Circ. Res. 97 (2005) 1232–1235.
[39] S. Krishnaswamy, W.R. Church, M.E. Nesheim, K.G. Mann, Activation of human prothrombin by human prothrombinase. Influence of factor Va on the reaction mechanism, J. Biol. Chem. 262 (1987) 3291–3299.
[40] C.E. Dempfle, Disseminated intravascular coagulation and coagulation disorders, Curr. Opin. Anaesthesiol. 17 (2004) 125–129.