Published in final edited form as:

Thromb Res. 2016 August ; 144: 106–112. doi:10.1016/j.thromres.2016.06.008.

Rivaroxaban improves patency and decreases inflammation in a mouse model of catheter thrombosis

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Abstract

Introduction—Dysfunction of indwelling central venous catheters (CVC) due to tissue ingrowth or clotting is common. The study objective was to determine if the oral anticoagulant rivaroxaban (RIVA) improved CVC patency and inflammation in mice.

Materials and Methods—Polyurethane catheters (0.5 cm length) were placed unilaterally into the external jugular vein (EJV) of mice, which subsequently underwent daily gavage with either vehicle or RIVA (5 mg/kg). CVC patency, as assessed by B-mode and Doppler ultrasound, and hematocrit were measured at 3, 7, 14 or 21 days (n=8-11 mice/group/time-point). Plasma monocyte chemotactic protein-1 (MCP-1) levels were assessed by ELISA, EJV matrix metalloproteinase-9 (MMP-9) levels by western immunoblotting, and cell proliferation (anti-Ki67), macrophage infiltration (anti-MAC387) by immunostaining of EJV tissues.

Results and Conclusions—CVC patency was significantly improved in RIVA-treated mice compared to vehicle-treated (93.8% vs. 62.9%) with the probability of patency in RIVA-treated mice being 1.5 times that in vehicle-treated (relative risk [RR], 1.50, 95% confidence interval [CI], 1.14-1.95, p=0.002). Plasma MCP-1 levels were lower in RIVA-treated mice vs. vehicle-treated at 21 days (389 ± 260 vs. 804 ± 292 ng/mL, p=0.005). Cell proliferation was less at day 7 in EJV from the RIVA-treated mice than vehicle-treated (5.0% ± 3.0 vs. 11.5% ± 3.6, p=0.0006), as were MMP-9 protein levels. There were no differences in hematocrit between vehicle and RIVA-treated groups at any time point. In conclusion, these data indicate RIVA lowers inflammation and

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Disclosure of Conflicts of Interest:

The authors have no conflicts of interest to disclose.

Addendum:

All authors designed the study, reviewed the manuscript and gave final approval of the manuscript. Y. He performed the experiments. Y. He and C.M. Terry performed analyses of raw data. C.M. Terry drafted the manuscript, Y. He edited the manuscript and A.K. Cheung provided critical input during all phases of the project, including conceptualization, study conduct, data analyses and manuscript preparation.

improves CVC patency in a mouse model, supporting future studies to assess RIVA for improving CVC patency in patients.

Keywords

Central venous catheters; chemokine CCL2; inflammation; rivaroxaban; venous thrombosis

Introduction

Central venous catheters (CVC) are essential and commonly used for a large number of medical indications. Unfortunately, CVC use is associated with a high incidence of complications, including thrombosis and occlusion. The intravascular placement of the catheter traumatizes the endothelium triggering activation of the coagulation pathways. In addition, the presence of the catheter stimulates foreign-body reactions and on-going inflammation. CVC function can be inhibited by formation of clots within the catheter lumen, mural thrombi, or thrombi at the tip of the catheter. CVC placement is also associated with an increased incidence of deep vein thrombosis (DVT), typically in the upper extremity. In a study of 157 chronic hemodialysis patients, the incidence of CVC thrombosis was 1.94/1000 catheter-days [1]. In the non-hemodialysis setting, long-term CVC use was associated with catheter-related thrombosis in up to 50% of pediatric patients and in up to 66% of adults [2]. Thrombi in CVC may also provide a colonization site for bacteria [3]. Given the common occurrence of thrombosis and far-reaching consequences associated with CVC dysfunction, strategies that further decrease thrombosis could have a large impact on patient morbidity and are urgently needed.

A number of approaches have been investigated to decrease CVC-related thrombosis, including changes in catheter design, novel catheter-surface coatings, use of catheter-locking solutions, and administration of systemic anticoagulants. Randomized controlled trials in hemodialysis patients have assessed the efficacy of systemically administered anticoagulants including fixed-dose and variable-dose warfarin, and low-molecular weight heparin (LMWH) [4]. Prophylaxis using mini-dose warfarin was not efficacious overall, but with post-hoc analysis improved CVC survival was observed if the international normalized ratio (INR) of >1.0 was maintained [5]. In contrast, in a larger double-blind randomized controlled trial, even with an INR target of 1.5-1.9, warfarin use was still not associated with improved CVC function [6]. Coli et al reported a significant decrease in CVC thrombotic complications in subjects administered ticlopidine and warfarin early after catheter placement compared to subjects given these agents after the first thrombotic event [7]. Meta-analyses of randomized controlled trials investigating the use of oral vitamin K antagonists or LMWH for preventing CVC-related thrombosis in cancer patients have in general not supported their use [8-10] although there is controversy [11].

Warfarin and LMWH have significant drawbacks. Warfarin requires dietary vigilance to avoid foods that alter vitamin K levels, its metabolism can vary significantly among patients and a number of common drugs alter its metabolism resulting in significant changes in warfarin levels. Thus warfarin use requires continued monitoring of INR. LMWH must be administered by subcutaneous injection and has been reported to cause thrombocytopenia in

0.2-5% of patients [12]. Of note, patients with heparin-induced thrombocytopenia were significantly more likely to have experienced a catheter-related DVT in the upper limb [13].

Rivaroxaban (RIVA) is an oral anticoagulant approved for the prevention of stroke in patients with atrial fibrillation, and for prophylaxis of DVT and pulmonary embolism after hip or knee replacement surgery. RIVA inactivates Factor Xa, a serine protease within the common coagulation cascade that converts prothrombin to thrombin. Thrombin converts fibrinogen to fibrin, which is the primary structural component of blood clots. Thrombin also activates platelets, triggering the release of growth factors that stimulate the proliferation of cells within the blood vessel wall and the release of other vasoactive agents such as vasoconstrictive prostaglandins. The critical role of thrombin in the activation of the coagulation cascade and in the release of growth factors and vasoconstrictive mediators suggests that inhibition of thrombin production by RIVA might be useful in improving CVC patency.

Herein, we report the use of a mouse model where a catheter section was permanently placed into the external jugular vein and used this model to evaluate the efficacy of RIVA on CVC patency.

Materials and Methods

Materials

Polyurethane catheters (0.84 mm o.d., 0.36 mm i.d.) were purchased from Braintree Scientific Inc. RIVA was purchased in powder form from Selleckchem. Mouse multi-analyte ELISArray kit was purchased from Qiagen. Enzyme-linked immunosorbent assay (ELISA) for MCP-1 was purchased from Peprotech and tribromoethanol (Avertin) was purchased from Sigma. Heparin as an unfractionated sodium salt was obtained from SAGENT Pharmaceuticals. Rabbit anti-human Ki67 (clone SP6) and rabbit anti-human Mac387 (clone MAC387) antibodies were purchased from ThermoFisher Scientific.

Mice

Mice with C57BL6 background (Jackson Laboratory) were allowed free access to food and water in a 12 h light/dark cycle. These studies were performed under the Institutional Animal Care and Use (IACU) guidelines and were approved by the University of Utah IACU Committee and the VA Salt Lake City Health Care System committee for animal care and use.

Catheter placement

Mice were anesthetized by intraperitoneal injection of tribromoethanol (200 mg/kg). After the skin was shaved and disinfected with topical 70% alcohol on the ventral side of the neck, a midline incision was made from the lower mandible to the sternum. Heparin (700 U/kg) was administered by intraperitoneal injection. A dissecting microscope with 5-fold to 45-fold magnification was used for the following surgical procedure. An external jugular vein (EJV), right or left, was randomly selected. The EJV was surgically freed from the surrounding connective tissue. The EJV was isolated by applying a vascular clamp on either

end. Dissection proceeded from the bifurcation at its distal end toward the proximal end as far as possible. A small venotomy was made with a 21-gauge needle in the distal region of the EJV. A 0.5 cm length of the polyurethane catheter, previously sterilized by soaking in 70% ethanol prior to use, was introduced into the venotomy and pushed in the caudal direction and completely embedded inside the vein. Once the catheter was completely within the vein, it was pulled in the distal direction, such that the venotomy through which the CVC was inserted, was blocked by the catheter itself (**Fig. 1**).

Sutures were placed on both ends to secure the catheter within the vein to prevent movement. The vascular clamps used to block blood flow were removed and the vein patency and hemostasis of the catheter were visually confirmed, then the neck incision was sutured closed.

Treatments

Mice were randomly assigned prior to surgery to either vehicle-treated or RIVA-treatment groups. RIVA was dissolved in phosphate-buffered saline (PBS), while PBS alone was used as the vehicle control. Beginning the day after surgery, gastric gavage using either RIVA (5 mg/kg) or PBS alone was performed daily until the predetermined endpoints. Animals were observed for bleeding from the surgical site and general physical appearance and activity. They were maintained for 3, 7, 14 or 21 days after CVC placement (n=8-11 animals per group per time point). Although patients typically receive a dose 20 mg/day which converts to ~0.25 mg/kg, a dose of 5 mg/kg of RIVA was used in this study to accommodate allometric scaling. This dose and higher doses have been previously used in mouse studies [14].

Ultrasound monitoring of CVC

Under anesthesia using isoflurane (1-5%) inhalation, the mice were affixed by tape in a supine position on a pre-warmed platform with the neck extended (**Fig. 2**).

After the skin had been shaved, ultrasound of the EJV was performed using a Vevo 2100 ultrasound machine (VisualSonics) with a 70-MHz transducer probe to check the blood flow through the catheterized section of the EJV. The position of the probe was optimized for B-mode images of the vessel wall, catheter wall and lumen interfaces (**Fig. 3**).

Color Doppler imaging of blood flow through the lumen was also performed and an example is shown in Supplemental Figures (**Supplemental Figure 1**).

After ultrasound assessment, the animals were euthanized and the vascular tissue was collected as described below.

Hematocrit and complete blood count (CBC) measurement. Serial systemic hematocrit measures were performed at three days, and one, two and three weeks after CVC placement and treatment initiation, as previously described [15]. Briefly, in sedated mice, the saphenous vein was punctured with a 23 g needle and the blood collected into a 40 mm heparin-coated hematocrit tube. The tube was sealed on one end then centrifuged for 120 seconds (StatSpin MP Centrifuge) and the packed cell volume was measured using a

hematocrit reader chart. At three weeks, the mice were euthanized and blood was collected by cardiac puncture. Anticoagulated (EDTA) blood was analyzed for CBC including hematocrit by an automated analyzer (Veterans Affairs Salt Lake City Healthcare System clinical laboratory).

Histology/Immunohistochemistry

The catheterized vein was dissected from surrounding tissue, explanted, rinsed with saline and fixed en bloc with 10% zinc-formalin. The vein including the catheter was embedded in paraffin and 5 μ m cross-sections were cut. Immunohistochemistry was performed using Vectastain ABC kit and Vector NovaRed Substrate Kit (Vector Labs). The tissue sections were heated at 95 °C with 10 mM sodium citrate buffer (pH 6.0) with the EZ-retriever microwave system (BioGenex) and treated with 1.0% hydrogen peroxide to block endogenous peroxidase activity. The sections were blocked with 5% normal goat serum in citrate buffer, then incubated overnight at 4 °C with the primary antibodies diluted at 1:200 for anti-Ki67 or 1:100 for anti-Mac387. The sections were washed and incubated with biotinylated IgG (1:200) for 30 minutes, followed with Vectastain ABC reagent, then with NovaRED substrate (Vector Laboratories) following manufacturer protocol. Finally, the sections were counterstained with Mayer's hematoxylin histological staining reagent (Dako). Relative quantification of IHC staining was performed using ImageJ (NIH, <http://imagej.nih.gov/ij/>). Immuno-positive cells and total cell numbers were counted in four different fields of view (680 $\times$ 512 pixels) per tissue slice. Other tissue sections were stained with hematoxylin and eosin, using standard procedures. Image Composite Editor (Microsoft Corp.) program was used to stitch the overlapping images of the longitudinal tissue sections.

Western immunoblot for MMP-9 protein

The EJV from vehicle-treated and RIVA-treated mice were harvested seven days after placement of the CVC. The catheter material was removed and the tissue lysed in standard RIPA lysis buffer supplemented with protease inhibitors (Sigma-Aldrich). EJV were also harvested from control animals with no CVC. Veins from three animals were pooled as one protein sample for each treatment group. Tissues were homogenized (Tissue Homogenizer, Omni International) and the homogenate was centrifuged at 8000 $\times g$ for 10 min at 4 °C. The supernatant was collected and protein concentrations were determined (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific). Protein (25 μ g) from each treatment group (RIVA-treated, vehicle-treated and no-CVC control) was loaded onto wells of a 4%-12% SDS-PAGE gel (Life Technologies) and separated by gel electrophoresis and transferred to 0.45 μ m nitrocellulose membrane (Life Technologies). After blocking (Tris-buffered saline containing 0.05% Tween 20 and 5% nonfat dried milk) for one hour at room temperature, the membranes were incubated with rabbit anti-human MMP-9 (1:1000 dilution; Cell Signaling Technology) overnight at 4 °C. The membranes were washed and incubated with horseradish peroxidase-conjugated anti-rabbit IgG antibody at 1:7000 dilution, washed, then bound antibody was detected with western blotting substrate (SuperSignal West Pico chemiluminescent substrate, Thermo Scientific), and exposed to X-ray film (Kodak). The densities of the bands were quantified and normalized against GAPDH, using image-analyzing software (ImageJ).

Enzyme-Linked Immunosorbent Assay (ELISA)

The euthanized mice underwent cardiac puncture directly after euthanasia and blood was collected into tubes pre-treated with EDTA. Plasma was collected by centrifugation at $6000 \times g$ for 10 min, then frozen at -80°C until further analysis. The Murine JE (MCP-1) ELISA Development Kit (PeproTech) was used following manufacture protocol to assess levels of monocyte chemotactic protein-1 (MCP-1) in the mouse plasma.

Statistics

One-way ANOVA with a Tukey post-hoc test was used to compare plasma MCP-1 levels between animals with CVC that were treated with RIVA or vehicle. An unpaired Student t-test was used to compare immunostaining data. Repeated measures ANOVA was used to test difference in hematocrit between the RIVA-treated and vehicle-treated groups. Relative risk (RR) for comparing the effects of RIVA-treatment versus vehicle-treatment on CVC patency was calculated using the Mantel-Haenszel and Chi-squared test.

Results

Rivaroxaban is well-tolerated in mice

A model of CVC dysfunction was successfully created with the placement of a catheter within the EJV of mice. The current model allows continuous blood flow within the CVC thus can be subjected to ultrasound assessment of drug effects on CVC patency. Animal physical activity, food and water intake, and general appearance after catheter placement appeared similar to those in un-operated mice, although these parameters were not specifically quantified. Physical activity, food and water intake, and general appearance were also similar between the RIVA-treated and vehicle-treated groups, suggesting the RIVA was well tolerated. No overt bleeding events were noted in mice treated with RIVA compared to vehicle.

Effect of RIVA on CVC patency and inflammation

A tendency for greater tissue in-growth in the lumen of the CVC in histology sections of the catheterized veins from the vehicle-treated animals was observed. However, tissue sections were often disrupted during preparation, with the CVC material being dislodged and pulling tissue from the slide. Examples of undisrupted histology sections are shown in **Fig. 4**.

The frequency of disrupted tissue prevented consistent quantitation of tissue in-growth within the lumen of the CVC. For this reason, CVC patency was only assessed by *in vivo* color Doppler US.

A greater number of CVC were patent at each time point in mice treated with daily RIVA, compared to vehicle-treated mice (**Fig. 5**).

For instance, in animals that had CVC in place for 14 days, 100% of CVC were patent in the RIVA-treated animals, compared to only 50% of CVC in the vehicle-treated group, as assessed by Doppler ultrasound. When analyzed *en toto* taking all time points into

consideration, the probability of patency in RIVA-treated mice was 1.5 times that in vehicle-treated mice (relative risk [RR], 1.50, 95% confidence interval [CI], 1.14-1.95, $p=0.002$).

Cell proliferation in the EJV was assessed by immunostaining tissue with the cell-proliferation marker Ki67. Cell proliferation was significantly decreased in tissue obtained from mice treated with RIVA versus vehicle-treated mice ($5\% \pm 3.0$ vs $11.5\% \pm 3.5$, respectively).

To assess RIVA effects on inflammation, the EJV tissue, collected from RIVA-treated and vehicle-treated animals at 7 and 14 days after CVC placement, was immunostained for presence of macrophages. No significant difference in macrophage infiltration into the tissue was observed between the RIVA-treated and vehicle-treated groups at day 7 or day 14 (not shown). However, the levels of plasma MCP-1, an inflammatory cytokine important in the foreign body response, were lower in RIVA-treated mice than in the vehicle-treated group at day 21 (**Fig. 6**).

Plasma levels of IL-6 or TNF- α were not significantly different (data not shown). MMP-9 protein levels were significantly decreased in catheterized vein tissue collected from mice treated with RIVA compared to untreated mice (**Fig. 7**).

Effect of RIVA on hematocrit and CBC

The hematocrit was measured at 3 days, one, two and three weeks after CVC placement and treatment initiation. At three weeks, the mice were euthanized and blood was collected for CBC including hematocrit. No significant difference in hematocrit values was observed between mice treated with RIVA or vehicle at any of the assessed time points (see **Table 1**). The hematocrit values obtained at three weeks by packed cell volume and by an automated analyzer were not significantly different. There was no significant difference observed between the vehicle- and RIVA-treated mice for any of the hematological parameters measured at 3 weeks by CBC (data not shown).

Discussion

We have developed a mouse model of CVC dysfunction. Others have reported rat and mouse models of CVC for removal of blood for repeat laboratory testing and for delivery of drugs. In these models, the CVC were inserted into the EJV, with the distal portion of the catheter subcutaneously tunneled then externalized at the dorsal side to be available for repeat access [16-19]. In another model, CVC were placed in the EJV of mice with the distal end connected to a pump that infused parenteral nutrition for up to 28 days [20]. In these previous models the EJV was ligated distally, so that return blood flow from the head in that vein was prevented. Patency was assessed by either drawing blood or injecting fluid through the CVC. However, Figueiredo et al assessed patency in a vascular access mini-port CVC mouse model using digital subtraction angiography [21]. The CVC in these previous models required repeat flushing with heparinized saline to maintain patency whereas ours did not. In Figueiredo's model, approximately 70% of CVC were patent at 7 ± 2 days and approximately 40% of catheterized vessels were patent at two weeks after implantation. In comparison, patency of CVC in the vehicle-treated mice in our study was 60% at seven days

and approximately 50% at two weeks. The CVC in our model experiences prolonged exposure to blood, similar to a CVC used for hemodialysis. However the model shares the characteristics of tissue in-growth and thrombosis common to all CVC, including those used for chronic drug administration and nutrition delivery. Also, we utilized Doppler US that does not require radiation exposure or injection of contrast dye for assessing patency.

In the current model, CVC patency in RIVA-treated mice was increased, compared to vehicle-treated. Plasma MCP-1 levels were significantly decreased in the RIVA-treated animals. These data support the notion that modulation of inflammation may be an important mechanism of action of RIVA, as well as its effects on coagulation. Others have reported in a mouse model of atherosclerosis and a rat model of stroke that RIVA treatment decreased tissue mRNA levels of inflammatory cytokines including IL-1 β , IL-6, TNF α and MCP-1 [22, 23]. Significant changes in plasma IL-6 or TNF α in RIVA-treated mice were not observed in our study. In proteomic analysis of plasma from patients randomized to warfarin or RIVA for 24 weeks, IL-6 levels were significantly elevated compared to baseline levels in RIVA-treated patients, while TNF levels were lower [24]. Thus, RIVA may have different effects on certain cytokines in mice compared to man but further studies are needed to confirm. We observed significantly decreased MMP-9 protein levels in the catheterized vein tissue of mice treated with RIVA (Fig. 9). Hara et al reported that RIVA treatment of ApoE-deficient mice for 20 weeks decreased MMP-9 protein in atherosclerotic plaques of the aorta [25]. In the previously mentioned proteomic study of human patients, plasma MMP-9 levels were also significantly decreased after 24 weeks of RIVA treatment [24]. Our findings illustrate that the changes in MMP-9 can occur fairly quickly as decreased MMP-9 protein occurred in vein tissue collected after only seven days of treatment. Also our MMP-9 and MCP-1 findings confirm findings by others in both mice and humans, further supporting that RIVA has effects on both coagulation and inflammation.

To our knowledge, the oral anticoagulants rivaroxaban, apixaban, and dabigatran have not been assessed previously in animal models or humans for the prevention of CVC-related thrombosis. However, in a randomized controlled trial of 8101 patients, RIVA was clinically superior to enoxaparin, a LMWH, at 35 days for reducing the risk of venous thromboembolism in hospitalized patients [26]. Placement of CVC significantly increases the risk of venous thromboembolism. Of note, there was a significantly increased risk of bleeding in the RIVA-treated patients. A small, unblinded, single arm trial is on-going to assess the effects of RIVA anticoagulation in cancer patients with an upper limb thrombosis associated with a CVC [Catheter-2, NCT01708850].

There was no significant difference in hematocrit between the RIVA-treated and vehicle-treated mice at any time point tested, suggesting that RIVA treatment significantly improved CVC patency without increasing occult bleeding. Additionally, there was no difference in any of the hematological parameters measured by CBC testing.

Dabigatran and RIVA are currently contraindicated in hemodialysis patients as these drugs are primarily cleared via the kidneys. However, the use of these agents in this population is steadily increasing. In one series, out of almost 30,000 hemodialysis patients with atrial fibrillation, 5.9% were started on dabigatran or RIVA [27]. Those initiated on RIVA had a

significantly greater relative risk of major bleeds compared to those initiated on warfarin (1.32 [95% CI: 0.93-1.87], $p = 0.03$), but in patients prescribed the lower dose of RIVA (15 mg/day), the risk of major bleeding was not increased. In a pharmacokinetic study, hemodialysis patients administered 10 mg RIVA had plasma drug concentrations that were similar to those in healthy volunteers given 20 mg [28]. Two large randomized clinical trials compared treatment with RIVA (30 mg/day for 3 weeks then 20 mg daily) and treatment with enoxaparin plus vitamin K antagonists for the prevention of DVT or pulmonary emboli. In pre-specified subgroup analysis of patients with mild or moderate-to-severe renal impairment in those studies, the incidence of venous thromboemboli was similar in both groups, but that the risk of major bleeding events was actually lower in the RIVA-treated group [28, 29]. These data suggest that RIVA may be as effective and possibly safer than standard anticoagulant treatment in patients with renal impairment. RIVA has fewer drug interactions than warfarin and there are no dietary restrictions with its use. RIVA does not require continued INR testing once the safe and effective dosing levels are established by evaluation of PT and PTT chemistries. But even with these benefits, further dosing and safety evaluations are required in hemodialysis patients before studies on the effects of RIVA on CVC-related thrombosis in the hemodialysis population should occur [30]. Some limitations of this work need to be acknowledged. We did not perform a direct comparison of RIVA with LMWH or warfarin, therefore we do not know if RIVA is more efficacious than those drugs in this model. We were not able to quantitate the effects of RIVA on tissue in-growth into the CVC since the tissue was often disrupted during histological preparation. Use of a larger animal model would make histological studies easier but a mouse model allows for the use of genetically modified animals and the direct assessment of the role of specific genes in CVC dysfunction.

Conclusions

This study has shown that RIVA administration is associated with significantly improved patency and decreased inflammation with no increase in bleeding in a novel model of CVC-thrombosis. The improved patency with RIVA administration may involve RIVA effects on both coagulation and inflammation. These findings support further studies on the use of RIVA to improve CVC function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Dr. Huan Li assisted with the ultrasound measures and Yingying Zhang assisted with statistical analysis. This investigation was supported by the University of Utah Study Design and Biostatistics Center, with funding in part from the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant 5UL1TR001067-02 (formerly 8UL1TR000105 and UL1RR025764). The work was also supported by the Western Institute of Biomedical Research, Salt Lake City, UT. The Small Animal Ultrasound Core Laboratory at the University of Utah was used for these studies.

Abbreviations

RIVA	rivaroxaban
CVC	central venous catheter
MCP-1	monocyte chemotactic protein-1
MMP-9	matrix metalloproteinase-9
DVT	deep vein thrombosis
LMWH	low-molecular weight heparin
INR	international normalized ratio
EJV	external jugular vein
ELISA	enzyme-linked immunosorbent assay

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Highlights

- Thrombotic complications are common with the use of central venous catheters (CVC).
- Warfarin and LMWH have drawbacks and uncertain benefits for preventing CVC thrombosis.
- Rivaroxaban has fewer drug and diet interactions and does not require therapeutic monitoring.
- Rivaroxaban improved patency and decreased inflammation in a mouse model of CVC thrombosis.

Figure 1.
Cartoon depicting placement of polyurethane catheter into the external jugular vein of mice.
Suture was placed at both ends of the catheter to secure the catheter in place.

Figure 2.
Vascular ultrasound of a catheterized jugular vein in a mouse.

Figure 3. B-mode US of a catheterized external jugular vein (JV). The central venous catheter (CVC) material appears hyperechoic compared to the native jugular vein and is indicated by brackets. The lumen of the JV is indicated.

Figure 4.

Histology of catheterized vein taken at various time points after CVC placement from vehicle-treated or RIVA-treated mice. Images “A, B, and C” are of histological sections obtained along the longitudinal axis of CVC within EJVs of vehicle-treated (A, B) or RIVA-treated (C) mice. The location of where the CVC walls were present within the EJVs is indicated by “CVC wall”. Tissue is present in the lumen of the CVC removed at 14 days after placement in (B). The images were reconstructed from multiple 4X histological images.

Figure 5.

Number of CVC that were patent at day 3, 7, 14 or 21 in RIVA-treated (+RIVA) and vehicle-treated mice, expressed as percent of total. Catheter patency was assessed at the indicated time-points by vascular ultrasound on sedated, live mice. N=7-11 for vehicle-treated and n=8 for RIVA-treated groups at each time point. Vehicle-treated (CVC) vs RIVA-treated (CVC+RIVA) (all days) RR=1.5, 95% CI, 1.14-1.95, p=0.002.

Figure 6.

Inflammatory monocyte chemotactic protein-1 (MCP-1) in plasma. Catheters were placed in mice and the mice were treated with vehicle (CVC), or rivaroxaban (5mg/kg) (CVC+RIVA) for the times indicated. Mice were sacrificed and MCP-1 levels in plasma were measured by ELISA. “No CVC, No RIVA” indicates plasma from unoperated, untreated mice (n=6). Day 14: n=10 (CVC), n=10 (CVC+RIVA); Day 21 n=10 (CVC), n=9 (CVC+RIVA). The F statistic from one-way ANOVA calculations was 8.7466 and p=0.000036. The p values from a Tukey post-hoc analysis are indicated by asterisks. The whiskers indicate the min and max values, the bar in the middle of the box indicates the median and the box indicates 1 SD from the mean.

Figure. 7.

Matrix metallo-proteinase-9 protein levels in control (uncatheterized) jugular veins (Control) or catheterized veins (CVC) from mice treated without (CVC) or with rivaroxaban (CVC +RIVA) for seven days. Protein homogenate from vein tissue collected at seven days after catheter placement was combined from three mice for each group. Protein was size separated on a polyacrylamide gel, transferred to nitrocellulose membrane and immunoprobed with anti-mouse MMP-9 antibody. Protein loading was assessed by immunostaining with anti-mouse GAPDH. Antibody binding was detected by chemiluminescence and autoradiography (image below the graph). The band intensities were measured by densitometry; the MMP-9 densitometry was corrected with the GAPDH signal (graph).

Table 1

Hematocrit

	Packed cell volume				Automated analyzer
Treatment group	3 day	7 day	14 day	21 day	21 day
RIVA	47.2 ± 7.3	44.5 ± 3.6	49.3 ± 4.6	46.3 ± 7.5 [*]	46.5 ± 5.7 [*]
vehicle	51.4 ± 1.8	48.1 ± 3.4	42.6 ± 4.9	44.2 ± 7.0 [*]	42.0 ± 10.1 [*]

^{*} p = ns, Paired t-test between hematocrit obtained at three weeks by packed cell volume vs automated analyzer. N = 8 mice per group.