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

1. 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, infection and stenosis development. 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

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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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.

2. Materials and methods

2.1. 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.

2.2. 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.

2.3. 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.

2.4. 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].

2.5. Ultrasound monitoring of CVC

Under anesthesia using isofluorane (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 Fig. 1).

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

2.6. 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 s (StatSpin MP Centrifuge) and the packed cell volume was measured using a hematocrit reader chart. At

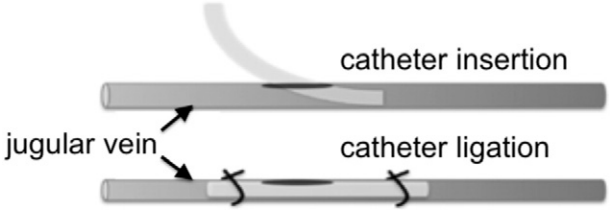

Fig. 1. Cartoon depicting placement of polyurethane catheter into the external jugular vein of mice. Suture was placed at both ends of the catheter to secure the catheter in place.