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Two swine models of iliac vein occlusion: Which form most contributes to venous thrombosis?

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ABSTRACT

Objectives: In the present study, we establish two swine models of iliac vein occlusion (IVO) with spontaneous thrombosis to understand the mechanisms linking IVO and thrombosis.
Methods: Two IVO models were established in 12 swine either by ligating the common iliac vein (CIVO) or both the common and external iliac veins (CEIVO). Venography was performed to assess each model and the associated thrombosis. Invasive blood pressure was also measured, and the vessels were examined histologically to analyse the pathological changes after ligation.
Results: On venography, the CIVO model showed common iliac vein (CIV) occlusion and reflux in the collateral veins whereas the CEIVO model showed occlusion in the CIV and external iliac vein (EIV), stasis in the EIV, and decreased collateral vasculature on venography. Thrombosis was only observed in the CEIVO model, which was with significantly higher venous blood pressure in the EIV and with significantly more thickened venous wall with lymphocytic infiltration histologically.
Conclusions: Two IVO models can be feasibly and reliably established in swine. The CEIVO model had a higher prevalence of thrombosis than the CIVO model. This CEIVO model produces comparatively less collateral drainage and greater inflammation that can contribute to the thrombosis prone to this type of model.

Introduction

Iliac vein compression syndrome (IVCS), also known as May-Thurner or Cockett's syndrome, is a condition characterised by varying severities of obstruction in the left common iliac vein, typically at the intersection of the iliac or hypogastric arteries [1–4]. IVCS is of clinical importance as it may cause blood outflow obstruction and reflux, leading to acute thrombosis and various forms of chronic venous disease (CVD). Patients with IVCS can be asymptomatic [5,6], which is because of the severity of the occlusion, drainage through collateral vessels, and other individual factors.

The pathogenesis of thrombosis in IVCS comprises three stages: asymptomatic vein compression, venous spur formation, and finally, deep vein thrombosis (DVT) [7]. Various venous experimental models to induce DVT have been described in the literature. Although a variety of approaches can be available to create such models, a more frequently used strategy is to produce a complete blood stasis by surgical ligation [8,9] or balloon occlusion of large vein [9–14]. Several adjunctive methods, including injection of extrinsic thrombin or ethanol, are usually necessary to increase the success rate of thrombosis. Other approaches, including electrical injury and photochemical reaction, are mostly used in mice models [15].

However, DVT is a complicated, multi-factorial process which can't be totally duplicated by each type of animal models. Currently used models of venous thrombosis fail to elucidate the exact role of collateral and occluded vessels in the progression of IVCS from venous spur to DVT formation. In the present study, we established two swine models of iliac vein occlusion, one model of common iliac vein occlusion (CIVO), and the other model of common and external iliac vein occlusion (CEIVO), to determine the roles of these different forms of occlusion in iliofemoral vein thrombosis.

Materials and Methods

This study strictly complied with the Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Animal Ethics Committee of Nanjing First Hospital, Nanjing Medical University.

Animal Model

All swine were purchased from the Laboratory Animal Center of Nanjing First Hospital. Twelve Hanford miniature swine were included

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in the study (six male, six female; age 16–20 weeks; weight 15–20 kg). The animals were randomly divided into two groups comprising six subjects each: Group A, which modeled CIV occlusion (CIVO); and Group B, which modeled CIV and EIV occlusion (CEIVO) (Fig. 1).

Anaesthesia was induced by administering 300 mg ketamine and 10 mg diazepam intramuscularly. Each subject was then continuously administered a suspension of 100 ml:5 g glucose injection and 20 ml:200 mg propofol via auricular vein for maintaining general anesthesia (1 ml/min). All surgical procedures were performed under sterile conditions.

Each animal was placed in a supine position on a digital subtraction angiography (DSA) table to allow fluoroscopic guidance during the procedure. Heparin was administrated intravenously during the operation to keep the activated clotting time above 300 s. The main purpose of heparinization was to avoid acute procedure-related thrombosis, because the creation of all models was performed under the guidance of fluoroscopy by keeping a guildwire in the vein served as a marker of left iliac vein. A 4F sheath (Cook) was inserted into the left femoral vein using the modified Seldinger technique, and a venogram (Omnipaque 350; GE Healthcare; Shanghai, China) was performed to visualise the iliac vein and inferior vena cava. The abdomen was incised at midline, and the left CIV and EIV were carefully exposed and isolated. In Group A, the proximal end of the left CIV was ligated with 4–0 silk (Ethicon, USA) (Fig. 1a). For subjects in Group B, both the left CIV and EIV were ligated together with 4–0 silk (Fig. 1b). The ligature sites were at the proximal end of left CIV and EIV, producing a segment of occlusion with a mean length of 3.55 ± 0.98 cm. Finally, a venogram was repeated to confirm that the iliac vein(s) was occluded; the abdomen was closed, and the skin was sutured with 4–0 silk. The animals were allowed to completely recover and permitted free access to water and food. All animals were administered an antibiotic (cefradine; 10 mg/kg; intramuscular) for 3 days postoperatively.

Invasive EIV Blood Pressure Measurement

During the ligation procedure, an invasive EIV blood pressure was measured before and after ligation of the iliac vein. A 4F pig-tail catheter (Cook) was advanced into the EIV through the sheath placed initially. The catheter was then connected to an electronic pressure transducer (Ultraview SL2700; Spacelabs Healthcare; USA) through a low compliance pressure line. The electronic transducer was calibrated to atmospheric pressure, and the invasive venous blood pressure was measured. However, EIV blood pressure measurement was not performed at 7 and 14 days after ligation, because EIV in Group B was full of thrombus and occluded.

Follow-up Venography

A venogram was repeated 7 and 14 days postoperatively. The procedures were performed under general anaesthesia as previously described. A 4F sheath was inserted into the left femoral vein, and the contrast agent (Omnipaque 350; GE Healthcare; Shanghai, China) was administered intravenously as described. Heparin was administrated intravenously during the venography to keep the activated clotting time above 300 s. Thrombus volume was also estimated on venography obtained on day 14, assuming that the thrombus was cylindrical.

Tissue Sampling and Histological Analysis

Three animals per group were euthanised at 7 and 14 days after ligation. The left CIV and EIVwere isolated and immediately fixed in formalin at 4 °C overnight. The tissues were then rinsed with distilled water, dehydrated through graded alcohol solutions, and embedded in paraffin. The venous tissue cross-sections (5 μm) were stained with haematoxylin and eosin (H&E) routinely.

Statistical Analysis

Data are presented as the range (mean ± standard error of the mean). Intra-and inter-group comparisons of the venous blood pressure were performed using the paired-samples *T* test. A value of $P < 0.05$ was considered statistically significant.

Results

Technical Feasibility

None of the animals died or experienced any procedure-related complications such as major haemorrhage or infection during the study period. The technical success rate of the model was 100% in both groups.

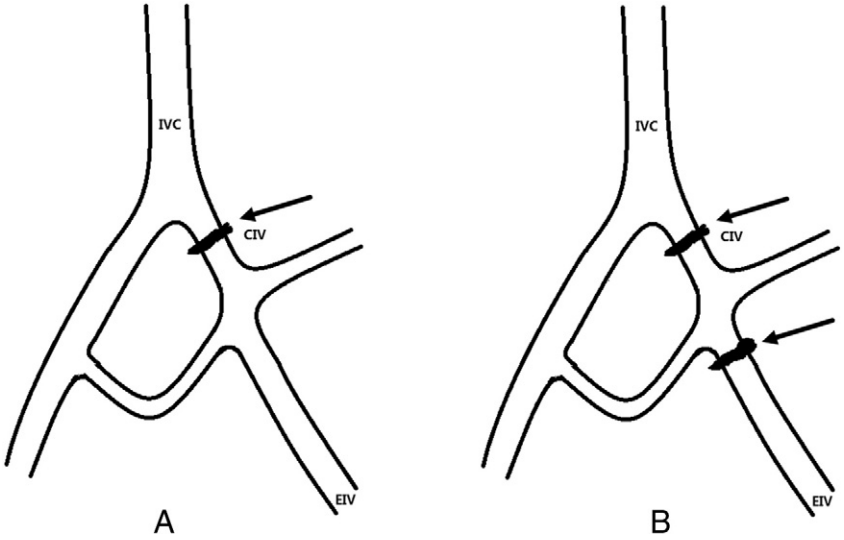

Fig. 1. Occlusion of the iliac veins in swine as a model of IVO.A, CIVO model created by ligating the CIV (arrow); B, CEIVO model created by ligating both the CIV (top arrow) and EIV (bottom arrow).IVO, iliac vein occlusion; IVC, inferior vena cava; CIV, common iliac vein; EIV, external iliac vein; CIVO, common iliac vein occlusion; CEIVO, common and external vein occlusion.