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Brief Communication

Evaluation of two experimental venous thrombosis models in the rat

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Introduction

A variety of animal models have been developed to assess the efficacy of antithrombotic agents, including experimental models of venous thrombosis [1–5]. A common feature shared by experimental animal models of venous thrombosis is a trigger of the thrombogenic state, which is usually composed of two components of Virchow's triad [6]. Either hypercoagulability or vessel injury combined with a reduced blood flow may be used to achieve a thrombogenic state. From the perspective of interlaboratory comparisons, the most problematic component of achieving a thrombogenic state seems to be hypercoagulability, which is usually triggered by an intravenous injection of recombinant or animal thromboplastin [1–4]. The activity of thromboplastin of different origin may vary considerably, which is clearly reflected by the high variation in thromboplastin dose required to achieve the hypercoagulable state in the animal experimental models of venous thrombosis [1–4]. Thus, the need for an in-house determination of the thromboplastin dose needed to achieve the hypercoagulable state is essential.

In the present study, two experimental venous thrombosis models in the rat were evaluated. The

Abbreviations: N, number of observations; S.E.M., standard error of mean; aPTT, activated partial thromboplastin time; TT, thrombin time; PT, prothrombin time; model 1, complete stasis combined with a hypercoagulability induced venous thrombosis model; model 2, partial stasis combined with a vessel injury induced venous thrombosis model.

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venous thrombosis model induced by complete stasis combined with a hypercoagulability (model 1) mimics the formation of in vitro clots. The second model of venous thrombosis induced by partial stasis combined with a vessel injury (model 2) allows some blood flow around the formed thrombi, but the absence of any specific exogenous trigger makes this model more comparable with clinical situation. The aim was to determine thrombogenic stimuli of a magnitude which would yield a thrombus of appropriate weight to study the antithrombotic effects of various agents. Thrombogenic stimuli, namely, hypercoagulability and vessel injury, were assessed by means of dose—response curves, while the complete (model 1) or the partial (model 2) blood flow occlusion was held constant in both experimental venous thrombosis models. Furthermore, in rats used in model 2, the daily haemostatic rhythm was investigated.

Materials and methods

Agents

Urethane and ferric chloride (both from Sigma-Aldrich, St. Louis, USA) were dissolved and diluted in distilled water. Rabbit brain thromboplastin (40 mg; Fluka Chemie, Buchs, Switzerland) was reconstituted in 4 ml of distilled water as prescribed by the manufacturer and diluted with saline just before use. Nadroparin (Fraxiparin®, 2850 IU anti-Xa/0.3 ml; Lek Pharmaceuticals d.d., Ljubljana, Slovenia) was dissolved and diluted in saline. Ketoprofen was used in the form of the formulation Ketonal® (100 mg/2 ml; Lek Pharmaceuticals d.d., Ljubljana, Slovenia). Pathromtin SL®, BC thrombin, Thromborel S®, Multifibren U® (all by Dade Behring, Marburg, Germany) and Auto Dimer® (Biopool Trinity Biotech, Wicklow, Ireland) were prepared as prescribed by the manufacturer.

Animals

Studies were carried out using overnight fasted male (model 1; 290—470 g, 3—4 months old) and female (model 2; 190—280 g, 3—4 months old) Wistar rats obtained from the Experimental Medical Centre of the Medical Faculty in Ljubljana, Slovenia. Fasted rats were used to facilitate surgical procedure, as the empty intestine gave more handling space for the isolation of the posterior vena cava. All animals received care in compliance with the European Convention on Animal Care. The protocol received the approval of the Veterinary Administration of the Republic of Slovenia with regard to the care and use of laboratory animals.

Complete stasis combined with hypercoagulability induced venous thrombosis model in the rat (model 1)

Thrombus formation was induced by a combination of complete stasis and hypercoagulability, as described by Vogel et al. [1]. Male rats were anesthetized with urethane (0.7 ml/kg of 20% w/v solution, intraperitoneally) and placed on a heated plate (38 °C) to maintain the body temperature. During anesthesia, the abdomen was opened by making an incision along the linea alba towards the sternum, followed by exposition of the posterior vena cava. Cotton threads, 2 cm apart, were placed caudally of the left renal vein loosely around the posterior vena cava to form a snare. All side branches of the posterior vena cava beneath the left renal vein and above the bifurcation of the posterior vena cava were ligated with cotton threads. Thrombus formation was induced by intravenous injection of 100, 300, 600, 1000 or 2000 μg/kg of thromboplastin into the right femoral vein, followed 10 s later by tightening two snares firmly around the posterior vena cava to induce blood stasis. Warm saline was sprayed over tissues, and muscle layer and skin were provisionally closed with bulldog clamps. Complete stasis was maintained for exactly 15 min. After this period, the thorax was opened, and a blood sample from the right ventricle was collected. Exactly 5 min after opening the thorax, the heart was incised, the ligated venous segment was excised, and the thrombus removed, blotted of excess blood and immediately weighed.

Partial stasis combined with vessel injury induced venous thrombosis model in the rat (model 2)

Thrombus formation by a combination of partial stasis and vessel injury was induced, as described by Gustafsson et al. [5]. Female rats were anesthetized with urethane (0.7 ml/kg of 20% w/v solution, intraperitoneally) and placed on a heated plate (38 °C) to maintain the body temperature. Before the surgical procedure, intramuscular injection of ketoprofen (35 mg/kg) was administered. During anesthesia, the abdomen was opened by making an incision along the linea alba towards the sternum, followed by exposition of the posterior vena cava. Partial stasis was induced in the posterior vena cava by tying a cotton thread