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Establishment of a canine model of acute pulmonary embolism with definite right ventricular dysfunction through introduced autologous blood clots

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ABSTRACT

Purpose: To establish a canine model of acute pulmonary embolism (PE) with right ventricular (RV) dysfunction using autologous blood clots and evaluate by echocardiography and contrast-enhanced Computed Tomography (CT).

Materials and Methods: Autologous blood clots formed *in vitro* were introduced sequentially into the pulmonary arteries of eight healthy mixed-breed dogs while monitoring pulmonary and systemic hemodynamic function. Blood clots were injected until the mean pulmonary artery pressure (MPAP) reached two–three times the baseline pressure, which was maintained up to 1 hour. The RV function was assessed by echocardiography and ECG-gated dual-source contrast CT.

Results: All animals survived the imaging procedure. The post-injection pulmonary angiograms showed extensive PE, and MPAP increased from 16.50 ± 2.45 mmHg to 43.13 ± 4.91 mmHg ($P < 0.001$). On echocardiography, the RV fractional area change decreased from 42.06 ± 3.36 to 27.96 ± 3.54 ($P < 0.001$), and the RV myocardial performance increased from 0.20 ± 0.05 to 0.63 ± 0.16 ($P < 0.001$). On CT, the RV end-systolic volume increased from 11.11 ± 1.81 ml to 24.71 ± 4.60 ml ($P < 0.001$), RV end-diastolic volume from 20.73 ± 2.83 ml to 34.63 ± 5.76 ml ($P < 0.001$), and the four-chamber RV/left ventricular diameter ratio from 0.38 ± 0.07 to 0.81 ± 0.14 ($P < 0.001$).

Conclusion: Acute PE with RV dysfunction was established in a large animal model through controlled injection of autologous blood clots, which may be useful for developing and evaluating new therapeutic approaches for acute PE with RV dysfunction.

Introduction

Right ventricular dysfunction (RVD) is a frequent consequence of acute pulmonary embolism (PE) and is a marker for increased risk of mortality in patients with acute PE [1–6]. Acute right-sided heart failure due to increased pulmonary vascular resistance and right ventricular afterload, which is caused by pulmonary artery obstruction and the release of vasoconstriction factors, is the primary cause of death in PE patients [7,8]. It has been suggested that acute PE patients with RVD have a worse prognosis [3–6,9–12]. In the International Cooperative Pulmonary Embolism Registry (ICOPER) study, the 90-day cumulative mortality reached 21% and 52.4% in patients with submassive PE and massive PE, respectively [12].

A relevant and appropriate large animal model of acute PE with RVD would be invaluable for development and evaluation of new drugs, devices, and methods for the diagnosis and treatment of this form of PE. Several experiments inducing massive PE in large animal models have been reported before [13–15]. However, the subjects were unstable owing to the risk of hemodynamic collapse, and right ventricular function was not evaluated. To our knowledge, a stable large animal model of acute PE with definite RVD has not been reported.

We aimed to create a canine model of acute PE model with definite RVD induced by autologous blood clots. The right ventricular function of the model was evaluated through hemodynamic monitoring, echocardiography, and contrast-enhanced dual-source Computed Tomography (CT) to confirm the RVD. This model will enable proper preclinical

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evaluation of various diagnostic and therapeutic approaches for treatment of acute PE with RVD.

Methods

All animal care and experimental protocols complied with regulations specified by the Guide for the Care and Use of Laboratory Animals (NIH Publication No.86-23, revised 1996) and the Institutional Animal Care and Use Committee of Nanjing Medical University. All procedures were performed on healthy mixed-breed dogs in Nanjing Medical University experimental laboratory by the skilled team of intensive care specialists.

Animal Model and Hemodynamic Measurement

Food was withheld from the animals for 12 hours prior to the experiment, and free access to water was available at all times. Eight healthy mixed-breed dogs (12–15 kg) of either sex were anesthetized with 3 mg/kg pentobarbital (Pentobarbital Sodium Salt, Chemical Reagent Company, Shanghai, China) administered intramuscularly. The trachea was intubated with spontaneous respiration. The anesthetic depth was regularly assessed by monitoring the photoreaction, corneal reflex, and respiratory rate (target range, 12–16 breaths/min). The subjects received 10 ml/kg of Lactated Ringer's fluid intravenously during the experiment. The left femoral artery was catheterized to enable measurement of the arterial blood pressure. In addition, a 16-F long catheter (Terumo Medical Corporation, Tokyo, Japan) was inserted into the right femoral vein and advanced until the tip reached the right atrium for injection of the preformed blood clots.

A 7-F pulmonary artery catheter (Swan-Ganz CCO mbo CCO/SvO2 7.5 F, Edwards Lifesciences, Irvine, CA, USA) was placed in the pulmonary artery via the right jugular vein under fluoroscopic guidance (Axiom Artis, Siemens, Munich, Germany). The correct location of the catheter was confirmed by detecting the typical pressure wave of the pulmonary artery. The catheter was connected to a Vigilance^{TM} monitor (Edwards Lifesciences) to continuously measure the cardiac output (CO). The catheter was also connected to pressure transducers to monitor the mean pulmonary artery pressure (MPAP), pulmonary artery wedge pressure (PAWP), and central venous pressure (CVP). The pulmonary vascular resistance (PVR) was calculated with the formula: $PVR = [(MPAP\text{-}PAWP)/CO)] \times 80$. The transducers were zeroed at the level of the right heart and recalibrated before each set of measurements. The heart rate (HR) was measured using a surface electrocardiogram (Philips, Eindhoven, Netherlands). Cardiac shock was defined as a mean arterial pressure (MAP) < 40 mmHg [16].

At the end of the experiment, the animals were euthanized with an intravenous overdose of pentobarbital and potassium chloride. Necropsy was performed, and the hearts and lungs were immediately explanted. The pulmonary arteries were examined to determine the location and morphology of the thrombosis.

Autologous Blood Clots Preparation

The autologous blood clots forming the embolus were generated using four rubber tubes (50 cm long with an approximately 5-mm inner diameter) and 50 ml of venous blood that was collected after placing the venous catheter. Each tube was connected to a three-way stopcock and infiltrated with bovine thrombin solution (500 IU/ml; Hunan Yige Pharmaceutical Co. Ltd., Hunan, China), followed by the blood until filled. The tubes were maintained at room temperature for 3 h, and the resulting thread-like clots were removed from the rubber tubes and washed three times in normal saline solution. The blood clots, which measured approximately 5 mm in diameter, were then cut into 10-cm long segments and drawn back into the tubes for later injection (Fig. 1A, B).

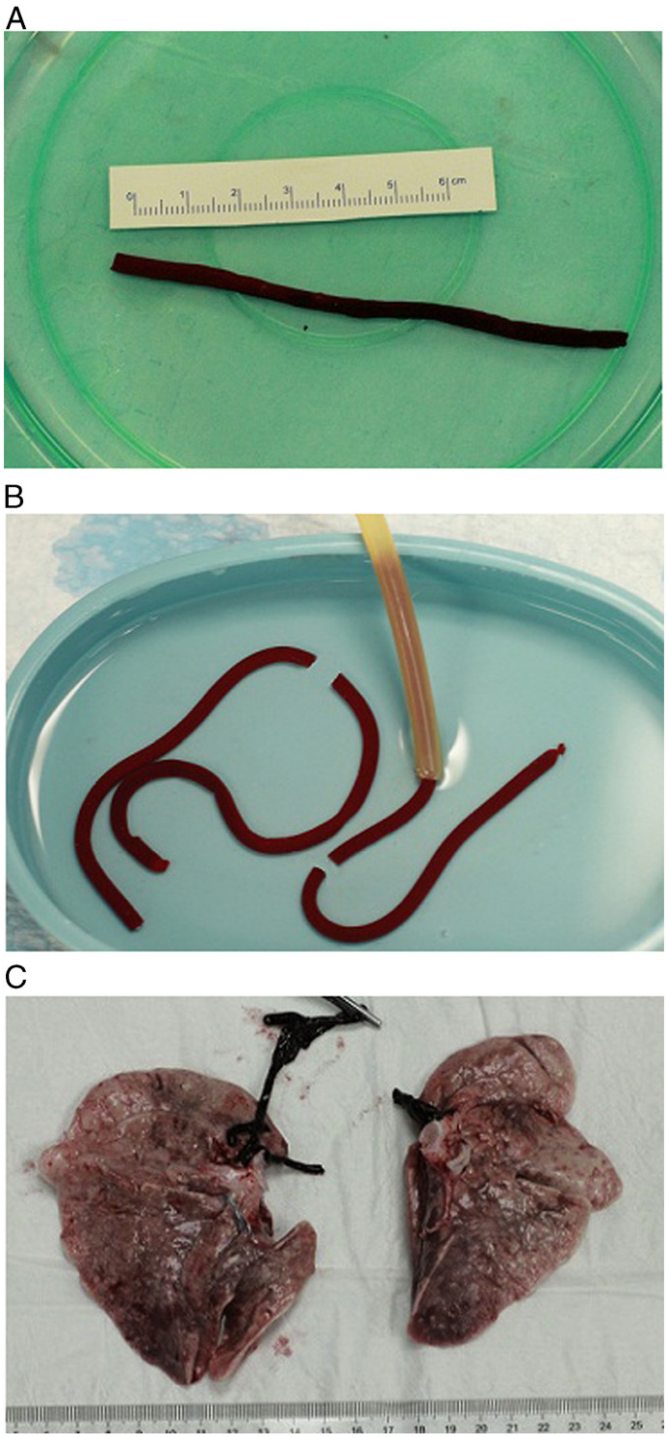

Fig. 1. Autologous blood clots *in vitro* and *in vivo* at the postmortem examination. (A) A blood clot approximately 5 mm in diameter was cut into segments measuring10 cm in length. (B) The clots were drawn back into the rubber tube for subsequent injection. (C) The postmortem examination of the animals confirmed significant embolization in both pulmonary arteries.

Experimental Protocol

Baseline data were recorded at least 60 min after inducing general anesthesia and placing all of the catheters. The subjects underwent baseline CT and pulse Doppler echocardiography examinations during this period, and the baseline pulmonary angiogram was performed using a