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Regular Article

New method of thrombus preparation using a fluid model for evaluation of thrombectomy devices in a swine model

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ABSTRACT

Background: Mechanical thrombectomy is a promising new modality of interventional stroke treatment. Preparation of thrombus is a very important step for the evaluation of the mechanical thrombectomy devices. The objective of this study was to explore a new method of thrombus preparation with fluid model (FM) for assessment of thrombectomy devices used in the recanalization of acute ischemic stroke.
Methods: Elongation test and catheter injection test were used to evaluate the mechanical properties of thrombi prepared by FM and static model (SM). Histological structures of two artificial clots and specimens of stroke patients were compared. Radiopacity of thrombus made by FM was evaluated in a swine embolization model.
Results: The maximum tensile length of thrombi prepared by FM and SM were significantly higher (4.28 ± 0.23 cm vs 3.16 ± 0.13 cm, $P < 0.01$) and showed less breakage on catheter injection test (13% vs 60%, $P < 0.05$). Histological features of thrombi prepared by FM showed mixed thrombus structure, similar to thromboemboli retrieved from acute stroke patients, while clots generated by SM were replete with erythrocytes. A total of twelve vessels in two swine were successfully occluded (TIMI 0 or 1), with sufficient radiopacity of each injected thrombus.
Conclusion: The thrombus prepared by FM had good mechanical stability, sufficient radiopacity, and similar histological structure of thromboemboli retrieved from stroke patients, which make it possible to be used in the evaluation of thrombectomy devices.

Introduction

Acute ischemic cerebrovascular stroke remains a severe disease despite improved outcome following intra-arterial thrombolysis. Recent studies have examined whether mechanical thrombectomy can accelerate revascularization and increase the revascularization rate. Thrombectomy devices such as a Solitaire flow restoration device and a Trevo retriever have been widely applied in the treatment of acute ischemic stroke [1,2]. The most common model used for the evaluation of the mechanical thrombectomy devices, is the swine model using thrombus made of bovine thrombin and barium sulfate. This thrombus however is a homogeneous erythrocyte-rich clot, which is likely to get fragmented during the procedure. In addition, its histological characteristics are not consistent with the thromboemboli of patients with acute stroke which is composed of multiple layers, including a fibrin-rich layer, a cellular component, and an erythrocyte-rich layer [3,4]. To solve this problem, Kan and his coworkers [5] developed a technique of thrombus preparation using whole swine blood mixed with 2 g of barium sulfate powder without the use of exogenous thrombin. They emphasized that their experimental thromboemboli using plain sedimentation were mechanically stable and histologically similar to typical thromboemboli retrieved from patients with stroke. Although their method was simple and easily controlled, the preparation condition was inconsistent with the flow environment in physiological blood circulation. In addition, the procedure was time-consuming.

To simulate the real conditions in human body, Roth et al. [6] prepared a thrombus using a modified method of the Chandler loop with the help of a dynamic clot-generating system. To our knowledge, however, the mechanical properties, radiopacity and histological features of this fluid thrombus model have not been reported so far. Therefore, the aim of this study was to describe a new technique of thrombus preparation with use of a fluid model (FM) to assess the

Abbreviations: FM, fluid model; SM, static model; LA, lingual artery; SCA, superficial cervical artery; DCA, deep cervical artery.

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usefulness of mechanical thrombectomy devices for acute stroke in a swine model. We also evaluated its mechanical stability, histological features, and radiopacity in the same model.

Materials and Methods

Animal care

The experiment was approved by the local animal care committee. Two swine (35-40 kg) were used in this study. The animals were fasted for 12 hours before angiography. Sedation was induced by a mixture of ketamine (8 mg/kg) and midazolam (0.3 mg/kg), and endotracheal intubation was performed. General anesthesia was maintained by 2% isoflurane inhalant. Vital parameters, such as arterial blood pressure, heart rate and expired oxygen and carbon dioxide levels, were continuously recorded. Then the right femoral artery was exposed surgically and a 7 F sheath (Cordis, Bridgewater, USA) was introduced into it. Autologous blood was obtained through the sheath before heparinization. After the experiment, an intravenous injection of 20 mmol potassium chloride was used to euthanize the animals.

Thrombus Formation

Preparation of fluid thrombus model

Autologous swine blood (10 mL) was extracted using a syringe from the 7 F sheath and poured into a plastic container with 1gram of barium sulfate. The plastic container was shaken for 10 seconds to mix the contents adequately. Bovine thrombin (25 IU) (MP Biomedicals, New Zealand) was added to the swine blood and mixed for 5 seconds. Six mL of the mixture was sucked into a 10-mL syringe and then slowly injected into a polyvinyl chloride (PVC) tube with 6 mm in inner diameter and 25 cm in length. During this procedure, care was taken not to introduce any air bubble into the PVC tube. The tube was coiled with the same material connector and set into a matching plexiglass ring as a rotation unit. Then, the rotation unit was mounted on a rotating shaft of the MR-16 blood clot detector (Sanhe Medicine, China) and slowly rotated for 15 minutes at a speed of 15 rpm. (Before the operation, the device was heated for 3 minutes until the thermostat pointer pointed at 37 °C). After the rotation, the generated thrombus was washed in physiologic saline solution, and was cut to a length of 20 mm and incubated at room temperature in a petri dish with saline for 30 minutes before application. A total of 15 sets were made by this method (Fig. 1).

Preparation of traditional static thrombus

As a control, a conventional experimental thrombin-induced thrombus was also prepared. The detailed preparation process is described elsewhere [3]. Briefly, 10 mL of swine whole blood was mixed with 25 IU of bovine thrombin and 1gram barium sulfate. Then, 6 mL of the mixture was drawn into a 10-mL syringe and then slowly injected into a PVC tube as described earlier. The tube was then place into a 37 °C incubator for 60 minutes. The clot was removed from the tube and cut to a length of 20 mm, which was incubated for 30 minutes at room temperature in a petri dish with saline before application. A total of 15 sets were made by this method.

Examination and evaluation of clots

Manual elongation test to measure thrombi tensile strength

The thrombus was placed on a tray covered by clean sterile towel at room temperature. Both ends of the 2-cm-long clot were grasped using forceps and slowly stretched until it fragmented. Then, the tensile length was measured into graduated scales. A strong tensile thrombus was defined as the clot which was stretched to more than one times of its original length [5].

Guiding catheter injection test to evaluate the elasticity and tenacity of clots

With the help of negative pressure produced by manual suction of a 50 mL syringe filled with physiological saline, the thrombus (diameter: ranging from 3.5 to 4.0 mm) was led into a PVC tube (inner diameter:4 mm) which was connected with the syringe. The PVC tube was connected to a 6-F guiding catheter after removing the air(Cordis, Bridgewater, USA). Then, gentle pressure was used to push the thrombus through the guiding catheter into the tray which was full of physiological saline. The breakage degree of clots after injection was recorded by digitized photos. Photoshop CS3 software was used to measure the length and diameter of thrombus before and after injection. This simple manual method was mainly used to evaluate the elasticity and tenacity of clots by simulating the process of thrombi injected into the target vessel in vitro. Forcing the thrombi through a smaller diameter 6 F guiding catheter could enumerate the discontinuity in the length

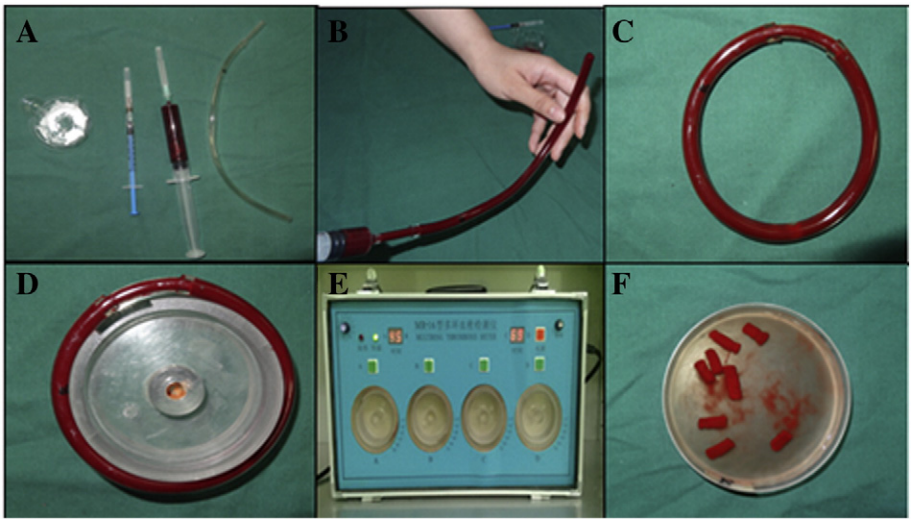

Fig. 1. (A-F) The process of thrombus prepared by the fluid model. (A) Note the materials of creating the artificial thrombus. (B) The mixture was slowly injected into a PVC tube taking care that no air bubble was injected into it. (C-D) Then the filled tube was coiled with the same material connector and this tube was set into a matching plexiglass ring as a rotation unit. (E) The rotation unit was slowly rotated in the MR-16 blood clot detector for 15 minutes at a speed of 15 rpm. (F) Thrombi incubated at room temperature (25 °C)in a petri dish with saline.