ORIGINAL ARTICLE

Development of a new model of transvenous thrombosis in the pig superior sagittal sinus using thrombin injection and balloon occlusion

Développement d’un nouveau modèle de thrombose veineuse du sinus sagittal supérieur chez le porc par ballon d’occlusion et thrombine

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Summary

\textit{Purpose.} — To establish an experimental model of superior sagittal sinus (SSS) thrombosis using a transvenous route, and thrombin and balloon occlusion, in pigs.

\textit{Methods.} — The SSS was catheterized transvenously in six pigs. Thrombin was injected into the pigs’ SSS to induce thrombosis. Magnetic resonance imaging (MRI) and magnetic resonance venography (MRV) confirmed successful SSS thrombosis. MRI and MRV were also used to observe the evolution of thrombus and accompanying brain parenchymal changes before thrombus induction postoperatively on Days 1, 3, and 7. The pigs were sacrificed for histological examination at the follow-up.

\textit{Results.} — SSS thrombosis was successfully achieved in all six pigs. On Day 1 postoperatively, MRV confirmed SSS thrombosis and MRI revealed brain edema in each animal. On Day 3, venous infarction was noted in two cases, one of which appeared to be hemorrhagic. On Day 7, MRV showed partial recanalization of the SSS in one pig. Brain edema was significantly relieved in four cases while, in two other cases, the extent of venous infarction was reduced. Histological examination confirmed SSS thrombosis in all animals, with recanalization in only one case. In...
Introduction

Cerebral venous and sinus thrombosis (CVST) is an uncommon disorder with variable clinical symptoms that range from no symptoms at all to severe venous infarction and concomitant morbidity [1]. However, although some progress in the pathophysiological understanding of this phenomenon has been made [2–7], complete physiological mapping of the event and the cause(s) of the accompanying brain parenchymal changes have yet to be elucidated. Also, the best treatment option for CVST remains unclear, given its low incidence and high clinical variability [8–10]. For this reason, several animal models of CVST have been developed to investigate the pathophysiological characteristics and accompanying brain parenchymal changes, as well the effect of treatment [3,4,7,11–14]. The current animal models, however, do not permit long-term follow-up, and are not suitable for pharmacological or mechanical recanalization, as sinus thrombosis induced by ligation and injection of thrombogenic substances does not resemble sinus thrombosis in humans. The aim of the present study was to develop a new model for superior sagittal sinus (SSS) thrombosis to investigate the pathophysiological mechanism(s) and to allow better comparisons of interventions and, ultimately, to find better therapeutic options for CVST.

Methods

This experiment was carried out in six healthy domestic pigs with a preferred type of venous connection between the internal jugular veins and distal spinal venous plexus, selected out of 12 pigs after assessment by cerebral (trans-carotid artery) angiography. The mean weight of the selected pigs was 30 ± 2 kg. The protocol was approved by the animal research committee of our institution, and was conducted in accordance with the guidelines of the International Council on Animal Care. The animals’ vital signs and physiological variables, such as arterial blood gas, pH values, blood glucose and rectal temperature, were monitored perioperatively, while neurological changes were routinely monitored and recorded daily by the animal experimental center and by ourselves.

Animal preparation

All six pigs were maintained on a standard laboratory diet, and their physical condition was monitored daily. After an overnight fast, the pigs were premedicated with an intramuscular injection of 1 mL of atropine, followed by an intramuscular injection of 0.2 mL of azaperone/kg body weight and 0.1 mL of ketamine/kg body weight. A solution of pentobarbital (30 mg/kg) was administered via an ear vein. Mechanical ventilation was performed throughout the entire experiment via an endotracheal tube. ECG monitoring was performed using vector ECG and the MRI scanner. Pentobarbital injections (1 mL each) were repeated as appropriate for the clinical state.

MRI protocol

With each animal, MRI was performed by a 3.0-T scanner (Achieva, Philips, The Netherlands) before and after thrombus induction to confirm sinus thrombosis. After thrombus induction, MRI was performed postoperatively on Days 1, 3 and 7 to observe occlusion and recanalization of the SSS, and any other brain-tissue injury. The imaging protocols included T1- and T2-weighted imaging, and magnetic resonance venography (MRV). All animals also underwent sagittal T1- and axial T2-weighted MRI.

The animals were placed in a prone position and fixed to the MRI table unit. After a brief gradient-echo survey scan, an initial MRI examination was performed using standard sequences, such as are used in imaging the cerebral sinus in humans. T2-weighted images were obtained in the axial view with the following parameters: field of view (FOV) 250 × 220 mm; time of repeat (TR) 3500 ms; time of echo (TE) 80 ms; thickness 3 mm; and flip angle (FA) 90°. T1-weighted images were obtained in the sagittal view with the following parameters: FOV 250 × 220 mm; TR 250 ms; TE 2.3 ms; matrix 280 × 194; 15 slices; thickness 3 mm; FA 90°. MRV was carried out with a PRESTO [multishot fast field echo—echoplanar imaging (FFE—EPI)] technique: TE > TR; TR/TE = 24/34; FOV 250 × 220 mm; slice 200; FA = 10°; TA = 04: 29. The maximum intensity projection (MIP) image was constructed, and cerebral venous sinus recanalization was assessed from both the MIP and original cross-sectional MRI scans.

Thrombus induction

After completion of the baseline MRI scans, the pigs were transferred to the angiography unit and placed in a supine position on the movable digital subtraction angiography (DSA) table. General anesthesia was maintained. After the limbs were straightened and fixed, femoral artery and
Femoral deep vein access was established under sterile conditions according to the following sequence. Initially, unilateral common carotid artery (CCA) and vertebral artery angiography was performed to acquire imaging of the arterial, capillary and venous phases. The communications between the venous sinus and internal jugular vein (IJV) were identified, and a 6-F guide catheter (Cordis Neurovascular, Miami Lakes, FL, USA) was advanced into the distal end of the right/left IJV. Retrograde venography was used to visualize the communications between the venous sinus and IJV. Using a coaxial technique, a guidewire-directed microcatheter (Echelon 10, EV3 MTI) was navigated into and lodged in the anterior third of the SSS under fluoroscopic guidance and the aid of venous road mapping via the communications between the venous sinus and IJV, transverse sinus and sinus confluence. A balloon catheter (Hyperglide, EV3, MTI) with a length of 15 mm and diameter of 5 mm was inserted and placed in the middle third of the SSS. The balloon was then inflated, and retrograde venography by microcatheter performed to confirm that the SSS had been completely occluded by the balloon. After this, the pigs were administered 100 U of thrombin (Sigma Diagnostic, USA) via a microcatheter within 15 min. The balloon was then deflated and unilateral CCA angiography performed to confirm thrombosis of the SSS. If no thrombosis was found, the balloon was re-inflated and additional thrombin administered by microcatheter to induce thrombosis once more. Once unilateral CCA angiography demonstrated thrombosis of the SSS, the microcatheter and balloon were carefully withdrawn. The introducer sheath was removed and manual pressure hemostasis carried out.

Histological examination

The pigs were killed by intravenous injection of an overdose of pentobarbital after systematic MRI examination. The SSS and both transverse sinuses were opened surgically to verify the extent of thrombus, and the thrombus, SSS and brain of each animal retained. After paraffin embedding, 5-μm-thick coronal sections were cut and stained with hematoxylin—eosin.

Results

Generation of the experimental model

The microcatheter and balloon catheter were both successfully navigated into and positioned within the SSS via the IJV, the communications between the venous sinus and IJV, transverse sinus and sinus confluence in all animals, while the thrombotic agents were delivered through the microcatheter (Fig. 1). Induction of SSS thrombosis was successfully achieved in all animals, as confirmed by unilateral CCA angiography immediately after the procedure and by MRV postoperatively on Day 1 (Fig. 2). During and after the procedure, no animal died due to complications.
related to the procedure nor did venous infarction result from the SSS thrombosis. In four animals, 100 U of thrombin was administered via microcatheter and the thrombus was formed within 15 min; in two animals, however, 150 U of thrombin was required and the thrombus took 30 min to form. Unilateral CCA angiography immediately after thrombus induction revealed retrograde flow and dilation of the bridging and cortical veins at the segment site of the SSS thrombosis.

**MRI and MRV**

Postoperatively on Day 1, MRV confirmed the presence of SSS thromboses in all animals, based on the typical signal loss at the site of thrombosis compared with the surrounding circulation. The thrombi demonstrated isointense/hypointense signals on T1-weighted images, and hypointense signals on T2-weighted images. Bilateral localized brain edema was also seen, with a slight hypointensity on T1-weighted images and hyperintensity on T2-weighted images (Fig. 3A, B). Cortical venous thromboses could not be identified on the applied MRI sequences and MRV. On Day 3 postoperatively, SSS thrombi persisted in all animals, as revealed by MRV. Bilateral localized brain edema in four animals remained stable, although venous infarction was noted in two cases (Fig. 3C, D). One of these cases appeared to involve hemorrhagic infarction, which was further confirmed by histological examination (Fig. 3C, D). Postoperatively on Day 7, MRV showed partial recanalization of the SSS in one animal (Fig. 2E). Bilateral localized brain edema was significantly relieved in four cases, and the extent of venous infarction reduced in two others.

**Discussion**

CVST is a rare, but important, cause of stroke that mostly affects young adults and children [15]. The clinical presentation and course of CVST is highly variable [1]. Symptoms vary from mild headache to severe intracranial bleeding and ischemic infarction, and the prognosis ranges from full recovery to severe disability and death [1,16]. Over the past decade, some progress in the pathophysiological mechanisms responsible for the wide variability of the clinical symptoms and course has been made [2—7]. The wide spectrum of symptoms and severity were deemed related to the extent and location of thrombus, venous collateral vessels and rate of thrombus progression [17]. However, the pathophysiological characteristics and cause(s) of the accompanying brain parenchymal changes of CVST are, as yet, not completely understood. The clinical reality is that the best treatment option for CVST remains elusive because of the low incidence and wide clinical variability of the disorder [8—10]. At present, anticoagulation is the most common first-line treatment, based on a single randomized controlled trial [18]. However, anticoagulation is not able to lyse the thrombus that occludes the sinus [19]. More recently, thrombolysis via intravenous or intra-arterial tech-
niques have been considered to be therapeutic options. The possibilities include direct intrasinus thrombolyis, mechanical thrombolyis and combined therapy to treat the CVST, and excellent results have been reported [10,19,20]. These published reports are limited to case reports, however, so it is not possible to determine whether or not these treatment options are superior to anticoagulation, nor which might be optimal [21]. An appropriate experimental model of CVST is needed to elucidate the uncertain pathophysiology of the CVST phenomenon and to allow preclinical comparisons of various therapies for CVST.

Several animal models (dogs, cats, pigs and rats) have been developed for the exploration of cortical and sinus venous thrombosis [3,4,7,11—14]. Occlusion of the venous sinuses is achieved by local injection of thrombogenic substances such as cyanoacrylate [11] and kaolin cephalin [7,14]. Permanent occlusion is achieved by ligation [2,4] or insertion of a balloon (12), and a hypercoagulable state is induced by intravenously administered homologous serum and total stasis of the sinus [22]. However, these methods are invasive and lead to iatrogenic brain parenchymal defects. In addition, during the process, the sinus has to be either opened or permanently ligated. Thrombosis induced in this way does not permit evaluation of the efficacy of new therapies, nor observation of the spontaneous course after reopening the occluded vessel or of the accompanying parenchymal changes.

In 2005, Rottger et al. [8,9], in light of the limitations of previous animal models, modified some of the methods described above. The team established a reversible model of SSS thrombosis in rats by removing the bone flap overlying the SSS and applying topical ferric chloride. However, removal of the entire length of bone overlying the SSS is traumatic and can result in infection. Also, the endothelial denudation induced by ferric chloride and aggregation of platelets is not in accordance with the pathophysiological characteristics of CVST in humans, where the initial cause of the CVST is not due to endothelial cell damage, but to a disorder of coagulation. In addition, the recanalization rate in this model is high and occurs within seven days. Thus, the method cannot be used for long-term mechanical studies.

In response to these limitations, Wang et al. [23] developed a reversible intracranial CVST model induced by slow injection of the thrombogenic agent via a microcatheter into the SSS. This model has the advantages of simulating the initial cause of the CVST and having a longer occlusion time, thereby allowing further detailed investigation of the pathological basis of thrombosis and evaluation of experimental therapeutic strategies (such as thrombolysis or anticoagulation). The major limitation of this method, however, is that it does not allow comparison of mechanical thrombolysis with other therapeutic modalities. In the present study, we have attempted to establish an augmented and clinically useful experimental model of CVST to investigate the pathophysiology of CVST and to allow better evaluation of various therapies for CVST.

For our study, we selected the pig to establish the SSS thrombosis model. Although others animals (cats, dogs and rats) have been widely used, the cerebral venous and sinus anatomy is rather irregular, with trabeculae and paranasules, in cats and dogs, and is very tiny in rats [24]. However, as pigs have an anatomy that more closely resembles that of humans, they are more suitable for studies of the pathophysiological mechanism(s) and comparison of various pharmacological therapies in CVST. Although the cerebral venous and sinus anatomy of the baboon most closely resembles that of humans, thus allowing intracranial venous retrograde access, primate models are difficult to justify from both ethical and economic standpoints. The cerebral venous and sinus anatomy of pigs is also closely similar to that of humans, except that the cerebral sinuses mainly drain into the spinal venous plexus and not the IJV [3]. Also, MRV studies of the cerebral sinus in pigs have revealed that they have an SSS with a mean diameter of 2.4 ± 0.56 mm [25].

Although an experimental model of SSS thrombosis in pigs has previously been developed, our present method differs significantly from the current optimal model [3,25]. Previously, the pig SSS thrombosis model was developed by combining balloon insertion and local injection of thrombogenic substances after surgical exposure of the SSS. However, because the cerebral sinuses drain into the spinal venous plexus and not into the IJV, based on the description by Fires et al. [3], access to the intracranial venous sinuses via a transfemoral/transjugular catheter approach appeared to be impossible.

However, prior to the present study, we performed cerebral venous angiography across the carotid artery in 12 mini-pigs and found that, in approximately 90%, there was a venous passage or communication between the IJV and distal spinal venous plexus. However, only around half these animals had a sufficiently direct and straight venous connection that would allow a microcatheter to enter the cerebral venous sinus. In most cases, the angulation of the venous passage would have prevented this, given the inability of the microcatheter and microguidewire to provide enough stress support for successful penetration. In the present study, the six animals studied were selected after undergoing transarterial cerebral angiography and evaluation. Thus, the microcatheter and balloon catheter could successfully be navigated into and lodged within the SSS via the IJV, allowing thrombotic agents to be delivered through the microcatheter. The duration of the procedure, from femoral puncture to thrombin injection, was 0.5—4 h, depending on operator experience and proficiency. However, the first procedure took around 4 h, whereas the last few pigs took only 1 h or less.

Compared with the currently used experimental model of SSS thrombosis, our method is minimally invasive. During the operations, there were no deaths or severe complications related to the procedure. In contrast, in the study of Stracke et al. [25], three out of 12 animals suffered acute subdural hemorrhage, one of which died during the intervention while another died after thrombus induction. In addition, our study used thrombin—currently available commercially—to induce thrombosis in the SSS, which changed the local coagulation state and initiated a true thrombotic process similar to the pathophysiological characteristics of CVST in humans. Furthermore, the SSS thrombosis is reversible, as spontaneous recanalization and venous infarction or hemorrhage were also observed in our model. Postoperatively on Day 3, venous infarction was noted in two animals, one of which appeared to be hemorrhagic. On Day 7 postoperatively, MRV showed partial recanalization of the SSS in one animal.
Our study, nevertheless, has several noteworthy limitations. First, the predisposing factor in human CVST is variable. The etiology includes puerperium, trauma, malignancy, disseminated intravascular coagulation, hypercoagulable states, infections, medications (such as synthetic steroids and contraceptive hormones), connective tissue disorders and dehydration, as well as several localized causes, such as brain tumors, arteriovenous malformations, head trauma, CNS infections, and infections of the ear, sinus, mouth, face or neck [1,16,26]. In the International Study on Cerebral Vein and Dural Sinus Thrombosis (ISCVT), 44% of patients had more than one identifiable cause or predisposing risk factor [16,26]. In our model, we only explored a single cause of CVST. The combination of injection of thrombogenic agent into, and balloon occlusion of, the SSS could only effectively produce a localized hypercoagulable state. The hypercoagulable state was transient and the system’s overall coagulation state remained virtually unchanged. This means that careful consideration should be taken of the differences in the natural history of the disease when the present model is used for research purposes.

Another limitation of our study is the small number of experimental animals used to develop the model. Also, the follow-up time was short for each animal. The rate of development of the SSS model by the transvenous route, using thrombin and an endovascular occlusion balloon, was influenced by the variations in the pigs’ venous anatomy, but how long the SSS thrombosis would persist after creating the model and what would be the recanalization rate over time remain unknown. Further experimental study and model development are required to resolve these unclarified issues.

The final limitation to the present study involves the technically demanding component of carrying out the study procedures. Navigating the microcatheter and balloon catheter to the SSS in pigs is difficult and requires training. To effectively replicate this work, a thorough knowledge of the cerebral venous and sinus anatomy of the pig, and a high level of proficiency in microcatheter manipulation, are essential.

We believe that the novel pig-based SSS thrombosis model we have developed and described here is the most similar to SSS thrombosis in humans so far. This model has the following potential applications: (1) it can be used for experimental imaging studies of cerebral sinus thrombosis to improve established scanning techniques or to develop new imaging techniques, such as molecular imaging using contrast media specific for human clotting; (2) it can be used to improve our understanding of the pathophysiology of the CVST phenomenon; and (3) it may lead to the development of better application and testing of therapeutic options for CVST in humans.

**Conclusion**

We have demonstrated that the development of an experimental model of SSS, using the transvenous route, thrombin and endovascular balloon occlusion, is feasible in pigs. This model most closely resembles SSS thrombosis in humans so far and is, therefore, the most useful for clinical applications. This model should allow for more effective studies into the pathophysiological mechanisms of CVST, more effective experimental imaging studies of CVST, and better testing and comparative studies of various clinical therapies for the condition.

**Conflicts of interests**

We declare that we have no conflicts of interest in this article.

**References**


