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Intraarterial Thrombolysis in a Pig Model: A Preliminary Note

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PURPOSE: To develop a pig model of arterial thrombosis suitable for assessing different methods of thrombolysis and to use this model to compare the efficacy of intraarterial thrombolysis performed by continuous proximal urokinase infusion versus mechanical clot disruption combined with intrathrombic urokinase injection. **METHODS:** In a control group of five pigs, a thrombus was made in a short segment of femoral artery and observed for 2 hours to assess its stability. In a treatment group of six pigs, intraarterial thrombolysis was performed immediately after thrombus formation. Thrombolysis was accomplished by continuously infusing urokinase into the proximal leading edge of the thrombus in three pigs and by mechanical clot disruption combined with intrathrombic urokinase injection in the remaining three pigs. **RESULTS:** There was no spontaneous reestablishment of flow in the control group during the 2-hour observation period. In the first treatment group, no flow was observed after a 1-hour treatment period when urokinase was infused continuously into the proximal edge of the thrombus. In the second treatment group, with mechanical clot disruption and intrathrombic urokinase injection, some degree of flow was observed in all three pigs. Reestablishment of flow was more sustained and of a greater degree with the addition of systemic heparinization. **CONCLUSION:** This animal model could provide a useful way to evaluate and compare different methods of thrombolysis. Our results suggest that mechanical clot disruption combined with intrathrombic urokinase injection is more effective in achieving reestablishment of flow than is continuous infusion of urokinase into the proximal edge of the thrombus.

Index terms: Animal studies; Thrombolysis; Interventional neuroradiology, models

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Intraarterial thrombolysis appears to be a promising technique for the treatment of acute stroke. Several case series have suggested that early reestablishment of flow in occluded intracranial arteries is associated with an improved outcome in stroke (1–5). At the present time, however, there is no consensus regarding which thrombolytic drug or method of intraarterial drug delivery is most effective in reestablishing flow. Procedural details, such as catheter type (end hole versus multiside hole), catheter position in relation to the thrombus, and delivery method (continuous infusion versus pulsed spray), remain controversial (6). Assessment and comparison of these particulars would be facilitated by testing in an appropriate animal model.

The purpose of this study was to develop a pig model of arterial thrombosis suitable for assessing different methods of intraarterial thrombolysis. The utility of this animal model is demonstrated in a comparison of the efficacy of intraarterial thrombolysis performed by continuously infusing a thrombolytic drug into the proximal surface of a thrombus versus thrombolysis achieved through mechanical clot disruption combined with intrathrombic drug injection.

Materials and Methods

In compliance with guidelines established by our institution's Research Animal Resources Center, Chester-

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White pigs weighing 20 to 40 kg were used for the study. Anesthesia was induced with an intramuscular injection of a cocktail composed of tiletamine hydrochloride/zolazepam hydrochloride combination (Telazol, Fort Dodge [Iowa] Laboratories Inc) (7 mg/kg), xylazine hydrochloride (1 mL/9.1 kg), and atropine (0.4 mg) and maintained with mechanical ventilation and inhalation of 1% halothane after endotracheal intubation.

Arterial Thrombus Model

A thrombus was created in a femoral artery of each pig by using a modification of a canine model of arterial thrombosis with endothelial injury previously reported by Badylak et al (7). First, a cutdown was created in the superficial femoral artery, and a 1.5-cm segment of artery just distal to the deep femoral branch was isolated with two silk ligatures. This location is an important feature of this model, as the deep femoral branch serves as a collateral pathway and prevents proximal propagation of thrombus. Next, all side branches arising from the isolated arterial segment were ligated except for one. This remaining side branch was used to cannulate the isolated arterial segment with a short piece of 3F tubing, which was used to drain blood from the isolated arterial segment. The arterial segment was then filled with boiling saline twice for 2.5 minutes each time. The proximal ligature was then loosened to allow the isolated arterial segment to refill with fresh blood. One hundred units of bovine thrombin (Sigma Chemical Co, St Louis, Mo) was simultaneously introduced into the arterial segment through the remaining side branch and allowed to mix with the fresh inflowing blood. The proximal ligature was then retightened, and the thrombus was allowed to mature for 30 minutes. The proximal ligature was removed after 30 minutes, and the distal ligature was released 15 minutes later, leaving a completely occlusive thrombus in the proximal superficial femoral artery. In each procedure, an electromagnetic flow probe (Carolina Medical, King, NC) was placed on the superficial femoral artery just distal to the thrombus site to measure flow within the artery both before and after thrombus production. The steps involved in producing this thrombus model are depicted in Figure 1.

In the control group of five pigs, the thrombus was observed for 2 hours to assess for spontaneous clot lysis and reestablishment of flow with the use of the electromagnetic flow probe to detect any blood flow distal to the thrombus site. After 2 hours, the arterial segment containing thrombus was excised. Three of the excised segments were opened longitudinally to examine the intraarterial thrombus grossly. The remaining two arteries were fixed in 10% formalin and processed through dehydration and paraffin infiltration. Each arterial segment was serially cut at 0.2-cm intervals and the entire specimen was embedded in paraffin. Transverse 5-μm-thick sections of paraffin-embedded arteries were placed on glass slides, stained with hematoxylin-eosin, and examined under a light microscope.

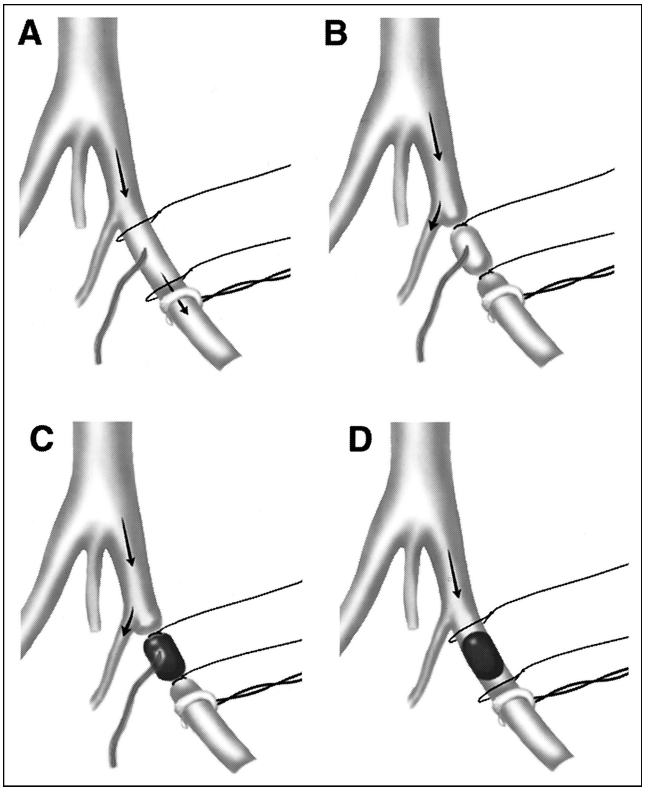

Fig 1. Steps involved in producing thrombus in the superficial femoral artery of a pig.

A, Short segment of superficial femoral artery just distal to deep femoral branch is isolated with two silk ligatures. Electromagnetic flow probe is placed directly on superficial femoral artery distal to isolated segment to monitor distal flow both before and after thrombus production.

B, Muscular side branch from isolated segment is used to introduce boiling saline followed by bovine thrombin. Deep femoral branch serves as collateral pathway.

C, After allowing inflow of fresh blood, segment is religated and clot is allowed to mature.

D, Ligatures are loosened, leaving short segment of artery completely occluded by thrombus.

Intraarterial Thrombolysis

In a treatment group of six pigs, intraarterial thrombolysis was performed immediately after thrombus formation. The thrombus was accessed by exposing the right common carotid artery and cannulating it with a 7F sheath. A 6F guiding catheter was then advanced to the external iliac artery under fluoroscopic guidance. Next, a Tracker 18 microcatheter (Target Therapeutics, Fremont, Calif) was passed in a coaxial manner to the thrombus site. Thrombolysis was performed through the Tracker 18 by administration of urokinase (Abbokinase, Abbott Laboratory, Chicago, Ill). The treatment group was divided into two subgroups: those treated with continuous proximal infusion of urokinase and those treated with mechanical clot disruption and intrathrombic urokinase injection.

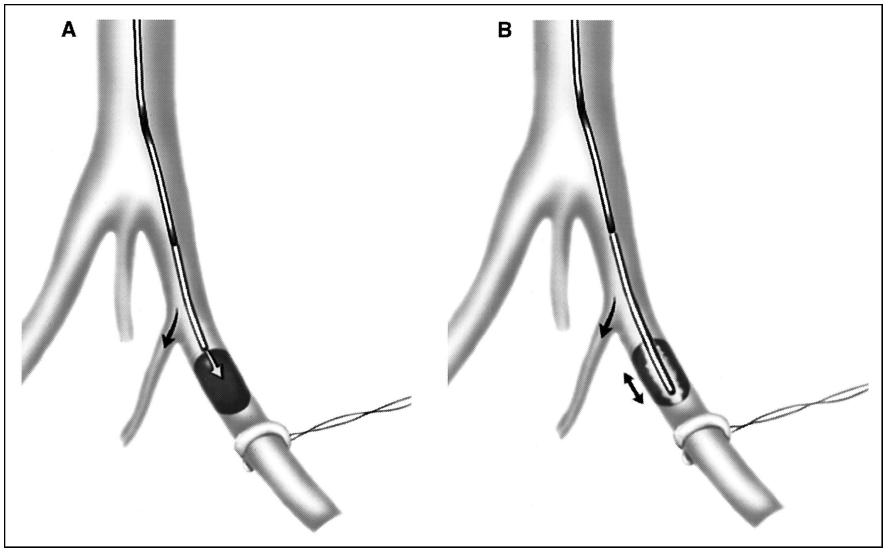

Fig 2. *A*, Illustration shows position of microcatheter for thrombolysis performed by continuous proximal urokinase infusion. In this group, guiding catheter was placed in iliac artery from carotid artery approach.

B, In mechanical clot disruption/intrathrombic injection group, microcatheter was passed back and forth through thrombus during urokinase injection. In both treatment groups, flow distal to thrombus was measured with electromagnetic flow probe during thrombolysis.

In the first three pigs, urokinase was continuously infused into the proximal edge of the thrombus over a 1-hour period using a volumetric infusion pump (Fig 2A). In pig 1, 250 000 U of urokinase diluted in D_5W to a total volume of 100 mL was infused during the 1-hour treatment period. In pigs 2 and 3, 500 000 U and 750 000 U, respectively, of urokinase diluted in D_5W for a total volume of 100 mL was infused over 1 hour. If necessary, the microcatheter was periodically advanced to abut the proximal edge of the thrombus. No heparin was administered to the pigs in this group.

Thrombolysis was achieved be means of mechanical clot disruption and intrathrombic urokinase injection in pigs 4 through 6 (Fig 2B). Mechanical clot disruption was done by passing a Taper-14 Flex-Tip microguidewire (Target Therapeutics) through the thrombus. The Tracker 18 was then advanced over the guidewire, and a bolus of 50 000 U of urokinase (50 000 U/mL concentration) was hand-injected distal to the thrombus. Afterward, the microcatheter was passed back and forth through the thrombus as boluses of 5000 U of urokinase were injected directly into the thrombus every 30 to 60 seconds. A guidewire was necessary to advance the Tracker 18 antegrade through the thrombus in most cases. Pig 4 was not given heparin; however, pigs 5 and 6 were treated systemically with a 50 U/kg heparin bolus followed by a 20 U/kg per hour continuous infusion delivered through the guiding catheter.

In each thrombolytic procedure, flow distal to the thrombus was recorded by using the electromagnetic flow probe placed directly on the superficial femoral artery. Periodic injections of contrast material through the guiding catheter served to confirm the flowmeter readings. In cases in which flow was reestablished, the degree of vessel patency was calculated as the percentage of baseline flow reestablished, with baseline flow being the flow volume in the artery before thrombus production as measured by the electromagnetic flowmeter.

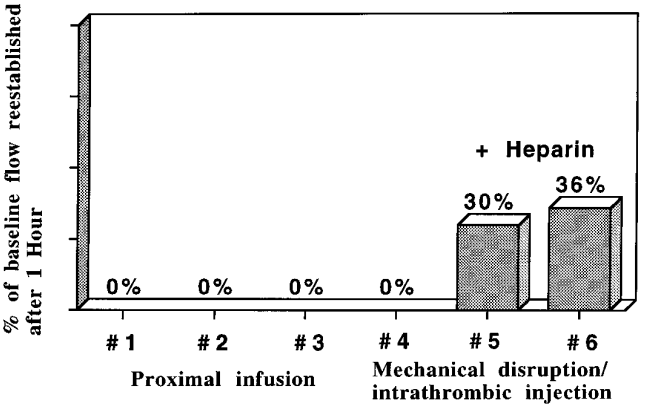

Fig 3. Graph shows percentage of baseline flow reestablished after 1 hour of thrombolysis. In continuous proximal infusion group (pigs 1 through 3), no flow was reestablished during or at end of treatment period. Some degree of flow was reestablished in all three pigs treated with mechanical clot disruption and intrathrombic urokinase injection (pigs 4 through 6); however, in pig 4, the artery reoccluded by end of treatment period. Only pigs 5 and 6 received systemic heparin.

Results

A completely occlusive thrombus was created in the superficial femoral artery of all 11 pigs used in the study. There was no evidence of spontaneous flow in the control group of five pigs during the 2-hour observation period, as measured by the electromagnetic flowmeter. Gross examination of the excised arterial segments revealed that the thrombi were soft and adherent to the arterial walls. Microscopic examination of cross sections of two arteries showed that the thrombi were composed pre-

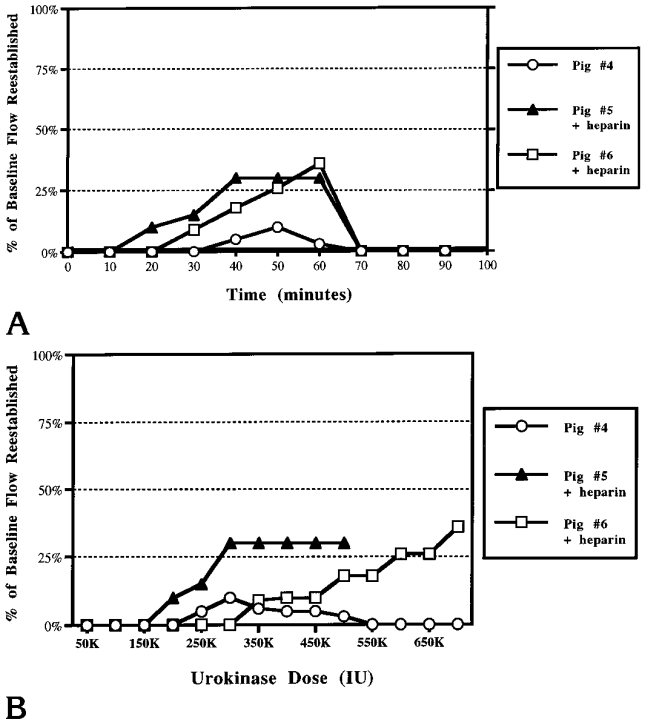

Fig 4. Flow versus time (*A*) and flow versus urokinase dose (*B*) in pigs 4 through 6, who were treated by mechanical clot disruption and intrathrombic urokinase injection.

dominantly of erythrocytes. No inflammatory or vascular reaction was observed.

The results of thrombolysis in the treatment group are summarized in Figure 3. No flow was observed during the 1-hour infusion period in the three pigs treated with continuous infusion of urokinase at the proximal surface of the thrombus. In fact, little fibrinolysis occurred in the proximal portion of the thrombus, as evidenced by inability to advance the infusion catheter very far along the thrombus site during the 1-hour treatment period in all three pigs.

Some degree of flow was reestablished in all three pigs treated with mechanical clot disruption and intrathrombic urokinase injection. However, in pig 4, who received no heparin, the artery reoccluded by 60 minutes and remained occluded despite continued injection of urokinase (Fig 4A). Only 11% of baseline flow was reestablished in pig 4 before the artery reoccluded. In pigs 5 and 6, who received heparin, 30% and 36% of baseline flow, respectively, was reestablished when urokinase injection was terminated at 60 minutes. Both these arteries reoccluded during the 10 minutes following the treatment period. Clearly, the addition of heparin appears to improve the degree of flow that is reestablished. Flow, as it relates to urokinase dose in pigs 4 through 6, is shown in Figure 4B. A dose ranging from 200 000 to 350 000 U urokinase was needed before flow could be detected distal to the thrombus.

Discussion

Several animal models of vascular thrombosis have been used to evaluate and compare different methods of thrombolytic therapy. Most recently, models of subacute venous thrombosis in the rabbit vena cava and canine iliac vein have been used (8–10). In both these models, thrombosis was induced by the introduction of stainless steel coils within the vessel lumen. The presence of a thrombogenic intravascular foreign body makes it difficult to use reestablishment of flow (the desired effect of thrombolytic therapy) as the measure by which different methods of thrombolysis are compared. In addition, the slow flow in the venous system does not adequately reproduce the conditions encountered when thrombolysis is performed in the arterial system. An in vitro model for assessing catheter-directed thrombolytic strategies has also been developed (11). This model is convenient and simplifies the multiple variables involved in thrombolytic therapy. However, thrombosis and fibrinolysis are complex processes that involve global interactions between hemodynamic and hematologic factors and between these factors and the arterial wall. These important components of thrombosis and fibrinolysis cannot be reproduced in an in vitro model.

The pig model used in our study is a modification of a canine model of arterial thrombosis previously described by Badylak et al (7). Comparison of different intraarterial thrombolytic techniques requires an animal model in which the thrombus is adherent to the arterial wall, because a nonadherent thrombus may form an embolism downstream in response to simple catheter manipulation or to the water-hammer effect of proximal arterial pulsations. Therefore, the effectiveness of different therapeutic techniques is difficult to assess when the thrombi are nonadherent. Producing a thrombus that is adherent to the arterial wall requires the presence of endothelial injury. This can be achieved by introducing boiling saline into the isolated arte-

rial segment in which the thrombus is made. Using scanning electron microscopy, Badylak et al (7) showed that this maneuver was sufficient to produce endothelial injury in their canine model. Damaged endothelium combined with stasis (caused by temporary artery ligation) and hypercoagulability (caused by introduction of bovine thrombin) contribute to the creation of thrombosis. Excessive proximal propagation of the thrombus is prevented by placing it just distal but close to the deep femoral artery, which, in our model, served as a collateral pathway.

We were able to establish a stable arterial thrombus in all 11 pigs used in the study. In a previous attempt to implement this model in canines, we were unsuccessful in consistently producing stable arterial thrombus, because the thrombi often spontaneously lysed and embolized downstream (unpublished data). This is presumably because the intrinsic fibrinolytic system in canines is very active, whereas the spontaneous fibrinolytic activity of pig plasma is much less active, more closely resembling human plasma in this respect (12, 13). On the other hand, in vitro studies have shown that plasma clots of pigs are more resistant to lysis with both urokinase and tissue-type plasminogen activator as compared with the plasma of humans and some primate species (14). However, these studies have also shown similar findings in both dog and rabbit plasma (14–16). Another limitation to consider when evaluating the results achieved with our pig model is that it is a model of acute arterial thrombosis in which the thrombus is composed predominantly of erythrocytes. It has been shown that platelet-rich arterial thrombi are much more resistant to thrombolysis than are erythrocyte-rich thrombi (17). Similarly, subacute and chronic thrombi, which are organized to different degrees, would be expected to be more resistant to thrombolytic therapy.

Several experimental and clinical studies have suggested that pulse-spray thrombolytic techniques can shorten overall treatment time relative to conventional, slow-infusion methods (8, 9, 11, 18–20). However, these studies were performed with the intent of applying different thrombolytic strategies to the treatment of lower limb and graft occlusions, and the treatment times with pulse-spray techniques were still relatively long. Moreover, these studies used delivery systems that are incompatible with the intracranial circulation. In our study, thrombolysis was performed with equipment that is readily available to any interventional neuroradiologist. There is general consensus that intraarterial thrombolysis should be performed within 6 hours of the onset of symptoms. Although thrombolysis may be successful beyond 6 hours, it may not be as safe. The 1-hour treatment time used in our study is within the time constraints imposed by most clinical situations. In our study, all three pigs treated with mechanical clot disruption and intrathrombic urokinase injection showed some degree of reestablishment of flow, whereas no flow was seen in the pigs treated with conventional infusion of urokinase at the proximal surface of the thrombus. However, it is difficult to apply these results to clinical situations. We did not evaluate whether mechanical disruption of the thrombus with the microguidewire and catheter caused clot fragmentation and distal embolization. If this were to occur clinically, small thrombi that embolize into distal branches of the intracranial circulation may become more difficult to lyse. The clinical outcome under such circumstances would depend on the particular branch embolized and the adequacy of pial collaterals.

The addition of systemic heparinization increased the degree of flow that was reestablished and appeared to prevent rethrombosis during thrombolysis in our pig model. Heparin has also been shown to improve the efficiency of clot lysis and restoration of blood flow in a canine model of arterial thrombosis (21). It seems reasonable to predict that systemic heparinization would improve the chances of recanalization during thrombolytic therapy in the stroke patient. However, the safety of heparinization during thrombolytic therapy from the standpoint of hemorrhagic complications remains unknown. Further investigation is required before the routine use of systemic heparinization during thrombolytic therapy in the stroke patient can be recommended.

In conclusion, we have described a pig model of arterial thrombosis suitable for assessing different methods of thrombolysis. Mechanical clot disruption in conjunction with intrathrombic urokinase injection seems to be much more efficient in reestablishing flow as compared with continuous urokinase infusion at the proximal surface of the thrombus in this pig model. The degree and duration of flow appear to be improved with the addition of heparin.

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