pISSN 1598-298X / eISSN 2384-0749
J Vet Clin 33(3) : 155-159 (2016)
http://dx.doi.org/10.17555/jvc.2016.06.33.3.155

Journal of Veterinary Clinics
www.ksvc.or.kr

Recombinant Tissue Plasminogen Activator Therapy for Aortic Thromboembolism in Four Dogs

Sei-Myoung Han, Ji-Ye Lee, Kyeong Kweon, Min-Cheol Choi*, Jung-Hee Yoon* and Hwa-Young Youn[1]

Department of Veterinary Internal Medicine, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea
**Department of Veterinary Medical Imaging, College of Veterinary Medicine, Seoul National University, Seoul 151-742, South Korea*

(Received: September 10, 2015 / Accepted: May 17, 2016)

Abstract : Four dogs were brought to the Veterinary Medicine Teaching Hospital of Seoul National University (VMTH SNU) with a history of hind limb ataxia, three with pain, one without pain. Three of the four showed weak to absent femoral pulses and cold extremities. Thromboembolism was identified by ultrasonography in the external and/or internal iliac arteries. A thrombolytic agent, recombinant tissue plasminogen activator (rt-PA), was administered (0.5-1 mg/kg, every 60-120 min, 3-5 doses). Two dogs (Cases 2 and 3), which were instantly provided rt-PA treatment, survived 6 and 17 months, respectively, although hematemesis and hematochezia were observed during treatment. In the other two dogs (Cases 1 and 4), rt-PA was administered 4 and 28 days after the appearance of pelvic limb symptoms, which may have limited the benefits of the treatment. When rt-PA treatment is instituted instantly and the side effects are monitored thoroughly during treatment, a good prognosis might be expected in canine aortic thromboembolism. For this reason, we suggest that rt-PA treatment should be initiated immediately if thromboembolism is identified.

Key words **:** Dog, Aortic thromboembolism, Recombinant tissue plasminogen activator, Thrombolytic agent.

Introduction

Aortic thromboembolism (ATE) is defined as acute thrombotic arterial occlusion. In feline patients, this disorder has been extensively studied and the typical clinical signs and causes are well described. Most cats have an underlying cardiac disease, with congestive heart failure reported in 40-66% of cats (9,10,14). However, in canine patients, the clinical presentation of ATE is less thoroughly documented. Hyperadrenocorticism, protein-losing glomerulonephropathy, infective endocarditis, and neoplasia are known to be underlying causes of aortic thrombosis in dogs (1,2,16). Hind limb paresis, absent femoral pulse, cold extremities, and signs of pain are typical clinical signs of ATE.

Recombinant tissue plasminogen activator (rt-PA) is a single-chain polypeptide serine protease produced by recombinant DNA technology, and is synthesized from cDNA obtained from a human melanoma cell line (17). This agent forms a complex between rt-PA and fibrin that preferentially activates thrombus-associated plasminogen, resulting in rapid fibrinolysis. Thus, it is known that rt-PA more effectively directed against the specific thrombus (18). For this reason, rt-PA, a thrombolytic agent, is preferentially used in dogs with ATE. However, the clinical use of rt-PA in dogs suffering from ATE has only been reported once in the published literature (3).

The current report describes the clinical signs and diagnosis of ATE in four dogs, as well as their treatment with rt-PA and the outcomes of the thrombolytic treatment. We also aimed to describe the use of the thrombolytic drug, t-PA, as a short-term therapeutic intervention.

[1]Corresponding author.
E-mail : hyyoun@snu.ac.kr

Case

Case 1

A castrated 9-year old male Schnauzer was referred to the Veterinary Medicine Teaching Hospital of Seoul National University (VMTH SNU) with a history of anorexia, vomiting, and melena.

A physical examination showed mild dehydration. The dog had a systemic blood pressure of 220 mmHg. A biochemical profile showed severe azotemia (BUN: 159 mg/dl; creatinine: 4.9 mg/dl), elevated liver enzyme levels (ALT: 481 U/L; AST: 105 U/L; ALP: 4373 U/L; GGT: 43 U/L) and electrolyte imbalance (Na^+: 145 mEq/L; K^+: 6.0 mEq/L; Cl^-:105 mEq/L). Hypercoagulability was identified with a shortened prothrombin time (PT: 5.1s) and shortened activated partial thromboplastin time (APTT: 4.8s). The urine was inadequately concentrated (specific gravity: 1.012) and proteinuria was detected [Urine protein creatinine ratio (UPCR): 5.25]. A radiograph and an ultrasound scan revealed hepatomegaly, splenomegaly, gallbladder mucocele, and renal cysts and calculi.

Based on these results, chronic kidney disease and protein-losing nephropathy were diagnosed and the following treatment started: amlodipine 0.1 mg/kg SID, PO; sylimarin 10 mg/kg, BID, PO; UDA 10 mg/kg, BID, PO; lefotil 1 T/day

BID, PO; omega 3, 2 ml/dose, BID, PO; aluminum hydroxide 30 mg/kg, BID, PO; kremezine 1 pouch/day, BID, PO; and fluid therapy.

On that day evening, ataxia developed in the hind limbs. Patellar luxation and hip joint dysplasia were not identified. After four days, non-specific and non-localizable pain developed. Using ultrasonography, a thromboembolism was identified in the left external iliac artery.

We decided to use thrombolytic therapy, namely rt-PA. Four doses of rt-PA (0.5 mg/kg at intervals of 120 min) were administered intravenously. Concurrently, hydromorphone (0.1 mg/kg, TID, IV) was injected for pain control. The following day, necrosis progression was detected in the left limb. The dog also developed dyspnea, which was followed by a cardiopulmonary arrest and resulted in death.

Case 2

A 14-year-old, spayed, female Shih-Tzu visited the emergency unit of VMTH SNU with symptoms of vocalization, dyspnea, and hind limb ataxia. One year previously, this dog had been diagnosed with mitral valve and tricuspid regurgitation and hyperadrenocorticism and had been treated with trilostane 3.5 mg/kg, BID, PO; benazepril 0.25 mg/kg, BID, PO; furosemide 3 mg/kg, BID, PO; and pimobendan 0.25 mg/kg BID, PO.

A physical examination revealed an absent femoral pulse in the left hind limb and cold extremities. The dog had a systemic blood pressure of 220 mmHg, although this measurement was not performed on the left hind limb. A biochemical profile showed elevated liver enzyme levels (ALT: 128 U/L; AST: 50 U/L; ALP: 4640 U/L; GGT: 85 U/L) and elevated creatine kinase (CK) levels (1494 U/L). The blood glucose level in the left hind limb was 64 mg/dl, while the systemic blood glucose was 140 mg/dl. The D-dimer value was 0.7 μl. An ultrasound scan revealed thromboembolism in the external and internal iliac arteries.

Treatment was started for aortic thromboembolism (ATE). Three doses of rt-PA (1 mg/kg at intervals of 120 min) were administered as thrombolytic treatment and hydromorphone (0.1 mg/kg, TID, IV) was injected for pain control. After the third administration of rt-PA, the treatment was stopped because hematochezia developed. The following day, pulses were restored and cold extremities had recovered in the hind limbs. Ultrasound scans confirmed the existence of thromboembolism lysis in the external and internal iliac arteries. Dalteparin, an anticoagulant, was prescribed for one month with a dosage of 150 IU/kg, TID, SC. However, The dog developed a cardiogenic pulmonary edema due to the pre-existing mitral valve and tricuspid regurgitation and died six months after the ATE diagnosis with no recurrence of thromboembolism.

Case 3

An 11-year-old, castrated, male Maltese was brought to the emergency wing of VMTH SNU with symptoms of non-specific pain, vocalization, dyspnea, and hind limb ataxia.

A physical examination found an absent femoral pulse. The systemic blood pressure was 130 mmHg. A biochemical profile showed mild azotemia (BUN: 43.7 mg/dl; creatinine: 1.1 mg/dl), elevated liver enzyme levels (ALT: 103 U/L; AST: 449 U/L; ALP: 281 U/L; GGT: 12 U/L), hypoproteinemia (total protein: 5.7 g/dl, albumin: 2.4 g/dl), and elevated CK levels (> 2000 U/L). The D-dimer value was in the normal range at 0.1 μl. An ultrasound scan showed thromboembolism in the bilateral external and internal iliac arteries.

To relieve pain, hydromorphone (0.1 mg/kg, TID, IV) and fentanyl patch 12 μg/hr were administered. Three doses of the thrombolytic agent, rt-PA (1 mg/kg at intervals of 120 min), were also administered to treat ATE. rt-PA administration was stopped after the third dose because hematemesis developed. The following day, dalteparin 150 IU/kg, TID, SC and clopidogrel 2 mg/kg, SID, PO, were prescribed for eight days and then the dog was managed with clopidogrel and/or aspirin 0.5 mg/kg, BID, PO.

About 40 days later, there was a recurrence of ATE. Anticoagulant therapy was restarted with Dalteparin 150 IU/kg, TID, SC and continued for 15 months, after which this dog was euthanized and autopsied with the consent of the owners. The autopsy showed that this dog had moderate, multifocal, suppurative endocarditis, pulmonary thrombosis, atherosclerosis, chronic multifocal lymphoplasmacytic interstitial nephritis, as well as renal tubular degeneration.

Case 4

A 13-year-old female shunauzer was brought to the emergency wing of the VMTH SNU with symptoms of non-specific pain, vocalization, dyspnea and hind limb ataxia. This dog had been diagnosed one year earlier with hypothyroidism, cholestasis, and cholelithiasis and had been treated with levothyroxine 0.015 mg/kg, BID, PO; sylimarin 25 mg/kg, lefotil 1 T/day; UDA 10 mg/kg, BID, PO; and SAMe, SID, PO.

A physical examination found the right femoral pulse was weak. The systemic blood pressure was 160 mmHg. The D-dimer value was in the normal range at 0.1 μl. Although this was strongly suggestive of thromboembolism, the ultrasound scan revealed that the cause was in fact, a decreased blood flow in the right external iliac artery.

For pain control, constant rate infusion of fentanyl 4 μg/kg/h and fentanyl patch (12.5 μg/h) was administered. Then, streptokinase 0.5 mg/kg, BID, PO and amoxicilline 22 mg/kg, BID, PO were prescribed to reduce vasculitis. During the treatments, the dog showed good vitality and gait with no complaints of pain or other symptoms.

Four weeks later, this patient visited the emergency unit again with the same symptoms of non-specific pain, vocalization, and dyspnea. An ultrasound scan revealed thromboembolism in the aortic bifurcation region.

We decided to administer five doses of the thrombolytic agent, Recombinant t-PA (1 mg/kg at intervals of 60 min). Constant rate infusion of hydromorphone was also given for pain control. The electrocardiogram was monitored although any coagulopathic symptoms (e.g., epistaxis, hematemesis, hematochezia, etc.) could not be examined during treatment. However, on the following day, the dog developed sudden dyspnea, followed by a cardiopulmonary arrest and subsequently, death.

Discussion

The age range of the four dogs in the present study was 9 to 14 years. They had concurrent underlying diseases such as protein-losing nephritis, hyperadrenocorticism, and hypothyroidism. An autopsy of Case 3 showed it also had moderate multifocal suppurative endocarditis. After thromboembolism was identified (Fig 1), rt-PA was used instantly for recanalization. Blood flow was re-established in Cases 2 and 3 (Fig 2) and the dogs survived 6 and 17 months, respectively. A summary of the clinical cases is given in Table 1.

ATE is rarer in dogs than in cats. ATE in dogs leads to weak or absent femoral pulses and neuromuscular dysfunction (cool extremities, hind limb pain, loss of sensation in the digits, hyperesthesia, and cyanotic nail beds) being variably present, while in cats, ATE usually causes acute and dramatic clinical signs secondary to tissue ischemia. For diagnostic purposes, thoracic radiography and complete echocardiographic examinations are important to identify whether heart disease might be present (5,11). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), lactate dehydrogenase, and CK levels might be elevated due to skeletal muscle ischemia and necrosis in the course of routine laboratory testing. The D-dimer test is an important diagnostic tool

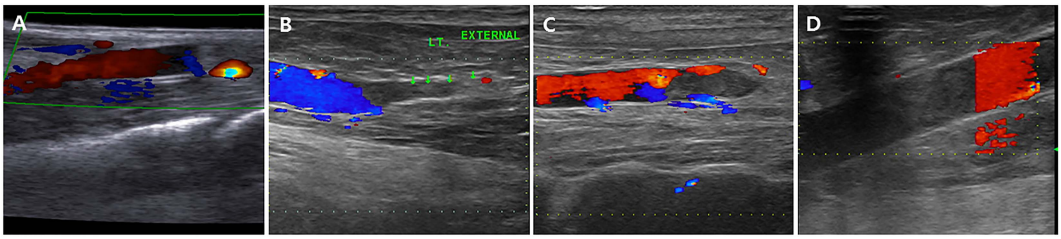

Fig 1. Identification of thrombus in four dogs by ultrasonography. The location of the thromboembolus was as follows: (A) case 1: left external iliac artery; (B) case 2: left external and internal iliac arteries; (C) case 3: bilateral external and internal iliac arteries; (D) case 4: aortic bifurcation region of the right femoral artery.

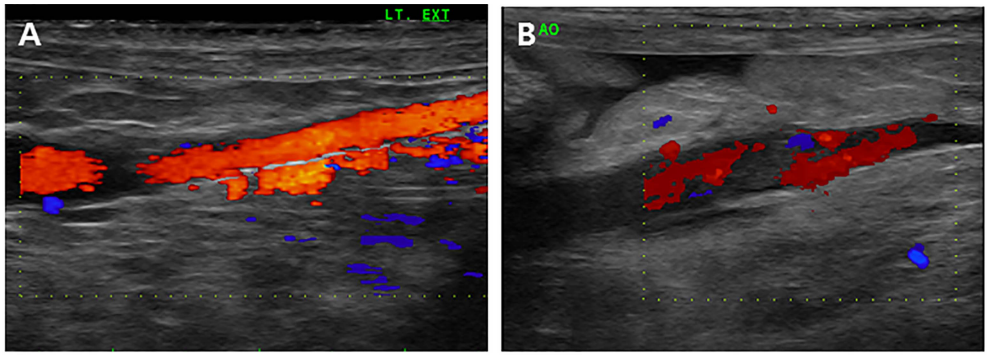

Fig 2. Identification of blood flow in two dogs after rt-PA administration. Blood flow was detected two and three days respectively after rt-PA was administered in Cases 2(A) and 3(B).

Table 1. Summary of clinical cases

		Case 1	Case 2	Case 3	Case 4
duration of pelvic limb sign before rt-PA administration		4 days	Acute onset	Acute onset	1 month
Clinical signs		Lameness	Paresis, paralysis	Paresis, paralysis	Lameness
Biochemical abnormalities		azotemia liver enzyme elevation electrolyte imbalance	liver enzyme elevation CK elevation	azotemia liver enzyme elevation electrolyte imbalance hypoproteinemia	NA
D-dimer (μl)		NA	0.7	0.1	0.1
rt-PA administration	Dose	0.5 mg/kg	1 mg/kg	1 mg/kg	1 mg/kg
	intervals	120 min	120 min	120 min	60 min
	times	4	3	3	5
side effects after rt-PA administration		NE	hematochezia	hematemesis	NE
survival after rt-PA administration		< 24 hr	3 months	12 months	< 24 hr
diagnosed disease		PLN	HAC	Neoplasia	Hypothyroidism

NA: not accessed; NE: not examined; PLN: protein loseing nephropathy; HAC: hyperadrenocorticism

for pathologic thromboembolism and has a high sensitivity but a low specificity (11). For this reason, it is important to interpret D-dimer results in the context of other clinical and laboratory test findings.

It is known that thrombosis in dogs is frequently a complication of an underlying disease such as immune-mediated hemolytic anemia, protein-losing conditions (4,6), hyperadrenocorticism (13), neoplasias (12), sepsis (8), early disseminated intravascular coagulation (DIC) and congestive heart failure (15). Spodsberg *et al.* (15) reported that fibrinolytic activity in dogs with either a systemic inflammatory condition or a protein-losing disorder was lower than in healthy dogs. One of the proposed mechanisms leading to hypercoagulability and increased risk of thrombosis in dogs with protein-losing conditions is enteric and renal loss of antithrombin (AT). Dogs with protein-losing enteritis and protein-losing nephropathy have decreased AT levels, which leads to an increased amount of thrombin, resulting in hypercoagulability (4,6). Hyperadrenocorticism has been reported to have the potential for both hypercoagulable and hypofibrinolytic states that can contribute to a prothrombotic state and thromboembolism (13). In neoplasms, tumor cells increase coagulation by platelet activation, thromboplastin release, and production of factor X activator. Tumor cells also reduce clotting factor clearance and neutralization, decrease fibrinolysis, interfere with the endothelial integrity, and disturb blood flow (12). Sepsis is associated with reduced activity of endogenous anticoagulants in dogs, suggesting an imbalance of hemostasis with increased thrombin-activatable fibrinolysis inhibitor activity in bacterial sepsis (7,8).

The purpose of ATE treatment is to stabilize the patient by providing supportive treatment and restoring perfusion by reducing the size of the thromboembolus and preventing extension of the existing thrombus and additional thromboembolus events. The therapeutic goal for ATE is antiplatelet and anticoagulant therapies to reduce platelet aggregation and growth of existing thrombi and the management of the underlying disease.

Rt-PA is a serine protease produced by recombinant deoxyribonucleic acid technology. Rt-PA and fibrin form a complex that preferentially activates thrombus-associated plasminogen, resulting in rapid fibrinolysis. Although fibrinolytic therapy to promote clot lysis with rt-PA was found to be the most potent therapy in this study, its use was limited due to the lack of clear dosage protocols. Though intensive care practices such as serum potassium concentration, acid-base status, and electrocardiographic monitoring are needed to prevent potentially serious complications, we were unable to closely monitor these four cases and could only examine their clinical signs (e.g., epistaxis, hematemesis, hematochezia, etc.). Rt-PA therapy is best instituted within 3 to 4 hours of vascular occlusion. Rt-PA has a half-life of 2-3 minutes in dogs and is cleared from their system within 5-10 minutes of stopping the infusion. Compared to other fibrinolytic agents such as streptokinase or urokinase, rt-PA exhibits significant fibrin specificity and affinity by binding to the thrombus and inducing a conformational change that facilitates the conversion of plasminogen to plasmin and dissolves the clot. For these reasons, rt-PA was selected as a thrombolytic agent to treat the dogs.

According to Clare *et al.* (3), rt-PA treatment for acute distal aortic thrombosis with bolus injections of 1 mg/kg every 60 minutes for a total of 10 doses was effectively perfused and a pulse could be palpated in one leg. In this study, three bolus injections of 1 mg/kg every 120 minutes efficiently perfused the vascular occlusion in all four cases. Cases 2 and 3, which instantly received rt-PA treatment, survived 6 and 17 months respectively, although they showed hematemesis and hematochezia during treatment. Cases 1 and 4 only received rt-PA treatment 4 and 28 days after the appearance of the pelvic limb symptoms, which may have limited the benefits of the treatment. Therefore, this study found that rt-PA therapy is most effective when administered 3 to 4 hours after ATE diagnosis and that the timing of rt-PA therapy is an important predictor of a good prognosis. For this reason, we suggest that rt-PA treatment should be initiated immediately if ATE is identified.

Conclusion

In two of the four cases discussed in this report, there was a gradual reduction in clinical signs after rt-PA treatment and survival of 6 and 17 months respectively. Treatment of rt-PA is successful in treating dog ATE if instantly administered. However, a thorough examination is warranted during the treatment because the development of complications is inevitable. When rt-PA treatment is provided instantly and any side effects monitored thoroughly during treatment, a good prognosis might be expected in dogs with ATE.

Acknowledgements

This study was supported by the BK21 Plus Program for Creative Veterinary Science and Research Institute for Veterinary Science, Seoul National University.

References

1. Boswood A, Lamb CR, White RN, Aortic and iliac thrombosis in six dogs. J Small Anim Pract 2000; 41: 109-114.
2. Carter AJ, Van Heerden J, Aortic thrombosis in a dog with glomerulonephritis. J S Afr Vet Assoc 1994; 65: 189-192.
3. Clare A, Kraje B. Use of recombinant tissue-plasminogen activator for aortic thrombolysis in a hypoproteinemic dog. J Am Vet Med Assoc 1998; 212: 539-543.
4. Donahue SM, Brooks M, Otto CM. Examination of hemostatic parameters to detect hypercoagulability in dogs with severe protein-losing nephropathy. J Vet Emerg Crit Care 2011; 21: 346-355.
5. Ettinger SJ, Feldman EC. Textbook of veterinary internal medicine. 2009: Elsevier Health Sciences.
6. Goodwin LV, Goggs R, Chan DL, Allenspach K. Hypercoagulability in Dogs with Protein-Losing Enteropathy. J Vet Intern Med 2011; 25: 273-277.
7. Jessen LR, Wiinberg B, Kjelgaard-Hansen M, Jensen AL, Rozanski E, Kristensen AT. Thrombin-activatable fibrinolysis inhibitor activity in healthy and diseased dogs. Vet Clin Pathol 2010; 39: 296-301.
8. Laforcade AM, Freeman LM, Shaw SP, Brooks MB, Rozanski EA, Rush JE. Hemostatic changes in dogs with naturally

occurring sepsis. J Vet Intern Med 2003; 17: 674-679.
9. Lake-Bakaar GA, Johnson EG, Griffiths LG, Aortic thrombosis in dogs: 31 cases (2000-2010). J Am Vet Med Assoc 2012; 241: 910-915.
10. Laste NJ, Harpster NK. A retrospective study of 100 cases of feline distal aortic thromboembolism: 1977-1993. J Am Anim Hosp Assoc 1995; 31: 492-500.
11. Nelson RW, Couto CG, Small animal internal medicine. 2014: Elsevier Health Sciences.
12. O'Keefe DA, Couto CG, Coagulation abnormalities associated with neoplasia. Vet Clin North Am Small Anim Pract 1988; 18: 157-168.
13. Park FM, Blois SL, Abrams-Ogg AC, Wood RD, Allen DG, Nykamp SG, Downie A. Hypercoagulability and ACTH-dependent hyperadrenocorticism in dogs. J Vet Intern Med 2013; 27: 1136-1142.
14. Smith SA, Tobias AH, Jacob KA, Fine DM, Grumbles PL. Arterial thromboembolism in cats: acute crisis in 127 cases (1992-2001) and long-term management with low-dose aspirin in 24 cases. J Vet Intern Med 2003; 17: 73-83.
15. Spodsberg EH, Wiinberg B, Jessen LR, Marschner CB, Kristensen AT. Endogenous fibrinolytic potential in tissue-plasminogen activator-modified thromboelastography analysis is significantly decreased in dogs suffering from diseases predisposing to thrombosis. Vet Clin Pathol 2013; 42: 281-290.
16. Sykes JE, Kittleson MD, Chomel BB, Macdonald KA, Pesavento PA. Clinicopathologic findings and outcome in dogs with infective endocarditis: 71 cases (1992-2005). J Am Vet Med Assoc 2006; 228: 1735-1747.
17. Thompson MF, Scott-Moncrieff JC, Hogan DF, Thrombolytic therapy in dogs and cats. J Vet Emerg Crit Care 2001; 11: 111-121.
18. Welch KM, Rozanski EA, Freeman LM, Rush JE. Prospective evaluation of tissue plasminogen activator in 11 cats with arterial thromboembolism. J Feline Med Surg 2010; 12: 122-128.