Comparison Between a Continuous Rate Infusion Protocol and an Accelerated Dosing Protocol Using tissue Plasminogen Activator for Thrombolysis in Cats and Dogs

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ABSTRACT

The aim of this study was to evaluate the short-term therapeutic effects and adverse events (AEs) associated with the use of tissue plasminogen activator (tPA) for the treatment of acute-onset aortic thrombotic disease (ATD) in cat and dogs. The ATD patients (13 cats and 5 dogs) with acute-onset hind limb paresis were categorized for either continuous rate infusion protocol (group A) or accelerated dosing protocol (group B), then divided further into low-dose and high-dose groups. Patients were randomly selected to receive tPA. Limb score and 48-hour survival rate did not differ significantly

between groups A and B (p = 0.0584 and 1, respectively). However, regardless of the method of administration, high-dose groups were superior to low-dose groups in regard to limb score reduction and 48-hour survival rate. AEs due to tPA administration occurred in 41.7% of patients who displayed azotemia, bleeding, reperfusion injury, and seizure. Finally, the percentage of patients who survived and were discharged was 12.5% in low-dose groups and 77.8% in high-dose groups. These findings lead us to believe that thrombolysis using tPA may be useful, depending on the treatment protocol.

INTRODUCTION

Cats are more prone to aortic thromboembolism (ATE) than aortic thrombosis (AT), and dogs are more prone to AT than ATE.[1,2] The majority of feline arterial thromboembolism is caused by cardiac disease.[3,4] In dogs, various underlying diseases such as cardiac disease, neoplasia, steroid administration, protein losing nephropathy, and hyperadrenocorticism may cause AT. When these underlying diseases are encountered, aortic thrombotic disease (ATD) occurs with an incidence of 0-50%.[5-12] If an ATD such as AT or ATE occurs acutely, the mortality rate is 55-73% for cats and 50-60% for dogs, and the median survival time for untreated dogs is as short as one day. Therefore, ATD treatment is needed.[6-9,11,13,14] Treatment methods for ATD include conservative management, thrombolytic agents, and thrombectomy, but no standard treatment method has been agreed upon.

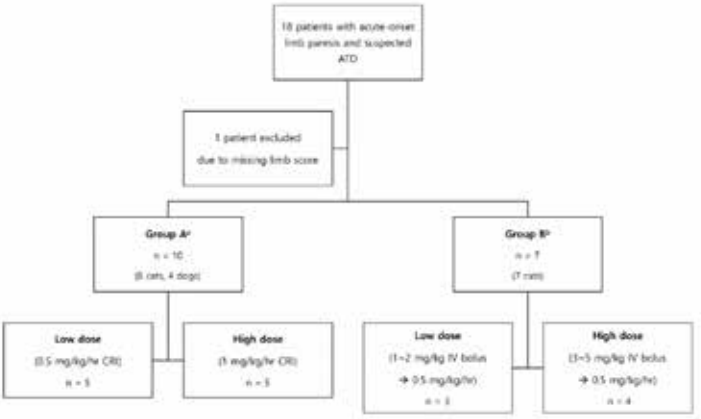
Recombinant tissue plasminogen activator (tPA) is a second-generation thrombolytic drug used in cats, dogs, and humans.[15] First-generation plasminogen activators such as streptokinase and urokinase bind to the circulating and fibrin-bound plasminogen to increase the risk of systemic fibrinolysis, but tPA binds only to the fibrin-bound plasminogen. Therefore, theoretically, tPA may be associated with a lower risk of bleeding and may be used specifically for thrombi.[16,17] The drug has a short half-life of 2 to 3 minutes, and is administered with continuous-rate infusion (CRI). The thrombolytic effects of tPA were related to dosage and concentration.[18]

It has been suggested that bolus dosing of tPA can require a high concentration within a shorter time to dissolve the blood clot as much as possible and reduce the risk of bleeding.[19] However, reduced bolus dosing in humans is not safer or more effective than CRI.[20] To compensate for the disadvantages of bolus dosing alone, the accelerated dosing protocol, in which a bolus dose was administered followed by CRI infusion, was used for the cats. However, the protocol resulted in poor outcomes.[14] Evidence regarding the use of tPA in cats and dogs is extremely limited because of the high cost and the concern of life-threatening adverse events (AEs), which may include reperfusion injuries, hemorrhage, hypotension, and signs of neurological problems.[21] Therefore, no treatment protocol has been established, and the dosage and method of administering tPA to cats and dogs vary.[14,22-24] The aim of this study was to evaluate the short-term therapeutic effects and AEs associated with the tPA administration for the treatment of acute-onset ATD in cats and dogs.

MATERIALS AND METHODS

From January 2015 to December 2017, this study was conducted on 5 dogs and 13 cats diagnosed with ATD among the patients who visited the referral veterinary hospital with acute-onset limb paresis. The diagnosis of ATD was made when the paresis of limb or limbs occurred suddenly, a palpable pulse was absent or weak, and there was pain and loss of warmth in the animal's extremities. Acute signs were defined as clinical symptoms that developed within the last 48 hours. All ATD patients were scheduled to receive tPA within 1 hour of their visits. The study participants were divided into two groups according to the administration method: those receiving CRI with a total of 20 mg for more than 4 hours (Group A), and those receiving an accelerated dosing protocol with a CRI of 0.5 mg/kg/h after the administration of a bolus dose for a total of 20 mg

Figure 1. *Flow chart of the study enrollment*



[a]*Continuous rate infusion protocol*
[b]*Accelerated dosing protocol*

(Group B). Each group was further divided into low-dose and high-dose subgroups (Fig 1). Patients were randomly assigned to each group. All participants received physical examinations, limb score evaluations, thorax radiography, and blood analysis (complete blood count, serum chemistry, blood gas analysis, coagulation test, cardiac troponin I, lactate) to evaluate their conditions. Each received analgesics and supportive care for underlying diseases. Chemistry and blood gas analysis were repeated according to the progress of treatment. To confirm the location of the thrombus, 16 patients were examined by echocardiography, abdominal ultrasound, computed tomography (CT), or brain MRI. Two patients did not undergo diagnostic imaging tests due to poor status. To quantitatively assess the recovery of limb function after tPA administration, a scoring system was used to measure each limb. The lower the value, the closer the limb function was to normal (1 = pulse and strong motor, 2 = pulse and weak motor, 3 = pulse but not motor, 4 = no pulse, no motor). During tPA administration, a continuous electrocardiogram (ECG) was performed to monitor for hyperkalemia. Patients were evaluated every 12 hours for 48 hours from the time of tPA administration in the intensive care unit to check for hemorrhage, signs of neurological problems, azotemia, hyperkalemia, acidosis, and ECG abnormalities. Each was assessed a limb score14 and survival time at baseline and 12, 24, 36, and 48 hours following the initiation of tPA. This study was terminated 48 hours after tPA administration. Each patient's treatment was then continued at the physician's discretion. Informed consent was obtained from all owners.

STATISTICS

Data were analyzed using statistical computer software (Prism 6 Version 6.01; Graphpad). Fisher's exact test was performed to compare data between each group. For all comparisons, a value of $p < 0.05$ was considered significant.

RESULTS

Eighteen ATD patients with acute-onset hind limb paresis for 2 years were included in the study. One was excluded from the study because the limb score could not be measured due to severe pain and the administration of propofol CRI. The mean age of the 4 dogs and 13 cats included in the study was 7 years (± 4.2). There were 13 males (2 intact, 11 neutered) and four females (one intact, three spayed). Domestic shorthairs (n = 3), Persians (n = 3), and Scottish Folds (n = 3) were the most common breeds. There were two Maltese, and one each of American Shorthair, Bichon Frise, Chinchilla, Russian Blue, Shih Tzu, and Turkish Angora.

The median duration to admission after the occurrence of ATD symptoms was 4 hours (range 1 - 48 hours). The most common underlying disease in cats that could induce ATD was hypertrophic cardiomyopathy (n = 8). There were two cases of chronic kidney disease, two cases of congestive

heart failure, and one case each of dilated cardiomyopathy, cerebral infarction, mammary gland adenocarcinoma, and idiopathy. There was one patient for which the underlying disease could not be diagnosed because the animal could not perform the diagnostic imaging test. After performing an abdominal ultrasonography or CT scan on 14 animals, thrombi were found in the blood vessels of 13 animals that could cause hind limb paresis:

- external iliac artery
- median sacral artery
- abdominal aorta
- aortic bifurcation
- aortic trifurcation, and
- abdominal terminal aorta

In one cat, no thrombus was detected.

Table 1 shows the limb score and the change of survival over time for each group. In both A and B groups, the limb score decreased. The survival rate was higher in the high-dose subgroups than in the low-dose subgroups after tPA administration. There was no significant difference in 48-hour survival between group A and group B, or between low-dose subgroup A and high-dose subgroup A ($p = 0.2063$-1). However, there was a significant difference between low-dose subgroup B and high-dose subgroup B ($p = 0.0286$). Patient 1 had no change in limb score during hospitalization, but limb score decreased to 1 point after one leg began to recover beginning the 9th day after tPA administration. AEs including reperfusion injury (n = 2), mucosal bleeding (n = 2), and seizure (n = 1) occurred in four of the low-dose subgroup A patients. In two patients with mucosal bleeding, the bleeding tendency was stopped by halving the rate of tPA administration. One patient with a reperfusion injury developed multiorgan dysfunction syndrome and was euthanized at the request of the owner 24 hours after tPA administration. One patient in high-dose subgroup A developed acidosis after tPA administration, but improved after receiving supportive care. In low-dose subgroup B, there were no AEs. In high-dose subgroup B, mild azotemia occurred after tPA administration in three patients. All of them improved after supportive care, but one also developed a seizure disorder. The patient remained under supportive treatment, and the limb score decreased. However, the seizures were so severe that the patient was euthanized at the owner's request 60 hours post tPA administration.

DISCUSSION

Because the clinical presentation, pathophysiology, and underlying etiologies of ATD differ between cats and dogs, it is difficult to uniformly evaluate the results of long-term survival follow-up.[1,5,7-9] Therefore, in this study, we evaluated short-term treatment effects and AEs only in patients with acute-presenting symptoms. There are few cases of tPA use in the treatment of ATD that occurs naturally in dogs, and there are cases with both favorable and negative outcomes.[9,10] Studies have shown that the range of survival after discharge for dogs with ATD is 50-60%, and the median survival time for acute cases is 9 days.[6-9] Two of the four dogs included in this study had decreased limb scores and 48-hour survival, but long-term follow-up was not included in this study, and the exact survival time remains unknown.

The survival rates of cats with ATD ranged from 39-45% for supportive care only, and 0-50% for thrombolytic treatment, regardless of drug type; 63.9% had 24-hour survival, and 27.3% survived until discharge after tPA treatment. It is reported that the use of thrombolytic agents has no survival benefit.[4,11,14,25,26] In this study, overall survival rates did not differ significantly from the previous study because 52.3% (9/17) of all patients – 50% (5/10) of group A, and 57.1% (4/7) of group B – survived for at least 48 hours. However, survival among the high-dose subgroups ranged from 80-100%, which differs significantly from previous studies.

The limb score was evaluated clinically because all patients included in this study

***Table 1**. Outcomes of 17 patients who received tPA administration for aortic thrombotic disease*

			Limb score					Survival					
		Patient	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h	Final	Adverse events
Group A	Low dose	1	6	6	6	6	6	O[b]	O	O	O	O	O	X[c]
		2	8	8	8	-[a]	-	O	O	O	X	X	X	O[d]
		3	8	8	-	-	-	O	O	X	X	X	X	O[e]
		4	8	8	-	-	-	O	O	X	X	X	X	O[e, f]
		5	6	6	-	-	-	O	O	X	X	X	X	O[d]
	High dose	6	4	4	4	3	2	O	O	O	O	O	O	X
		7	8	8	-	-	-	O	O	X	X	X	X	X
		8	6	6	6	6	6	O	O	O	O	O	O	O[d]
		9	3	3	3	3	3	O	O	O	O	O	O	X
		10	8	6	4	2	2	O	O	O	O	O	O	X
Group B	Low dose	11	8	8	-	-	-	O	O	X	X	X	X	X
		12	8	8	-	-	-	O	O	X	X	X	X	X
		13	5	5	3	-	-	O	O	O	X	X	X	X
	High dose	14	8	4	4	3	2	O	O	O	O	O	O	O[g]
		15	8	4	4	2	2	O	O	O	O	O	O	X
		16	4	2	2	2	2	O	O	O	O	O	O	O[g]
		17	8	7	6	6	6	O	O	O	O	O	X	O[d, f, g]

[a]*The patient died, and the limb score could not be measured.*
[b]*Yes.*
[c]*No.*
[d]*Reperfusion injury.*
[e]*Bleeding.*
[f]*Seizure.*
[g]*Azotemia.*

had hind limb paresis. There was no increase in limb score after administration of tPA. The limb scores decreased in 20% (8/10) in group A and 71.4% (5/7) in group B, but there was no significant difference in limb score ($p = 0.0584$). There was no decrease in low-dose subgroup A. The limb scores of two patients in high-dose subgroup A decreased, and all survived. In low-dose subgroup B, the limb score decreased in only one dog, and all patients died (3/3). In high-dose subgroup B, the limb scores decreased in all four animals and all four survived for 48 hours. In both groups, 12.5% (1/8) of the low-dose subgroups and 66.7% (6/9) of the high-dose groups displayed a decrease in

limb score, showing that the higher the dose, the higher the fibrinolysis effect, resulting in a significant limb-score decrease ($p = 0.0498$). Within the low-dose subgroups, 12.5% (1/8) had 48-hour survival; within the high-dose subgroups, 88.9% (8/9) had 48-hour survival. Thus, a higher dose resulted in a greater decrease in limb score and an increase in survival rate ($p = 0.0034$).

The severity of the patients was assessed using the limb score, with 8 points being the most severe. Five patients with a limb score of 8 were included in both group A and group B. We compared the reduction of the limb scores and the 48-hour survival rate for patients with the most severe forms of disease. There was no difference between group A and group B ($p = 0.5238$), but there was a difference between low-dose subgroups and high-dose subgroups ($p = 0.0476$). In fact, in the high-dose subgroups, the limb score decreased and four out of five had 48-hour survival, but in the low-dose subgroups, none of the patients had a limb-score decrease and none survived for 48 hours. These results are similar to the results of a thrombosis model study using beagles, and reaffirms that the thrombolytic effects of tPA are dose-dependent.[18,27]

We presumed that high doses of tPA may increase the incidence of AE in a dose-dependent manner, but occurrence of AE was not dose-dependent when compared between low-dose and high-dose subgroups ($p = 1$). In this study, AEs after the administration of tPA included azotemia, bleeding, reperfusion injury, and seizure. Azotemia occurred in three patients in group B, all of whom had 48-hour survival. One of them had severe seizures and was euthanized 60 hours after tPA administration. In this study, two patients had seizures and died. Three out of four reperfusion injury cases died. Mucosal bleeding occurred in only two patients in low-dose subgroup A, and both of them died. In low-dose subgroup A, all patients with AEs died, and in low-dose subgroup B, no AEs occurred, but all died. High-dose subgroup A had only one patient with an AE, and that patient did not die. In high-dose subgroup B, AEs occurred in three patients, but all survived for 48 hours. It is difficult to judge an AE as a negative prognostic factor, and further study is needed on the effect of AEs on survival.

There are some limitations to this study. Because there was no control group, we compared the results of previously published studies and only studied short-term survival. In addition, the number of participants in this study was low because it was expected that the incidence of AEs of tPA would be high. Thus, long-term survival studies involving control groups and larger studies are needed to improve accuracy.

This study suggests that the higher the tPA dose, the higher the thrombolytic effect and the short-term survival rate. However, due to the high cost of tPA, it may be difficult to use many patients in practice. Therefore, it is necessary to study the dosing regimen which can cost-effectively maximize thrombolytic effect of tPA.

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