Optimal strategy for effective dissolution of the thrombus in thrombolysis models

Young Dae Kim

Department of Medicine

The Graduate School, Yonsei University

Optimal strategy for effective dissolution of the thrombus in thrombolysis models

Directed by Professor Ji Hoe Heo

The Doctoral Dissertation
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

Young Dae Kim

June 2012

This certifies that the Doctoral Dissertation of Young Dae Kim is approved.

_____

Thesis Supervisor: Ji Hoe Heo

_____

Thesis Committee Member: Myoung Hee Kim

_____

Thesis Committee Member: Yong Rok Kim

_____

Thesis Committee Member: EungYeop Kim

_____

Thesis Committee Member: Jong-Baeck Lim

The Graduate School

Yonsei University

June 2012

Acknowledgments

I would like to express my gratitude to all those who gave me help for completing this study. I am deeply appreciated to my mentor and supervisor Prof. Ji Hoe Heo for stimulating suggestions and encouragement in all the time of research and writing. I gratefully acknowledge helpful comments and suggestions by Prof. Myoung Hee Kim, Prof. Eung Yeop Kim, Prof. Jong-Baeck Lim, and Prof. Yong Rok Kim. I would also like to express a special gratitude to Dr. Il Kwon who advised and encouraged me as a colleague, and sometimes as an younger brother. At last, I would like to give many special thanks to my parents and wife (Mi Ae Kim) and my sons (Ye-Jun and Ye-Suk) whose love enabled me to complete this work and live in this world.

Finally, I simply hope that my devotion to medical science could one day open doors to those who seek genuine help.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Optimal strategy for effective dissolution of the thrombus in thrombolysis models

Young Dae Kim

Department of Medical Science

The Graduate School, Yonsei University

(Directed by Professor Ji Hoe Heo)

Thrombolytic treatment with intravenous (IV) recombinant tissue plasminogen activator (rt-PA) is a proven therapy for acute ischemic stroke. However, its efficacy is limited due to its relatively low potency in dissolving thrombus, as well as the likelihood for hemorrhagic transformation and re-occlusion. During thrombolytic treatment with rt-PA, the thrombus undergoes dynamic changes. Lysis of the thrombus alternates with re-growth of the thrombus, leading to re-occlusion and platelets play major roles in this process. We investigated whether the combined use of aggrastat or atorvastatin can potentiate the efficacy of rt-PA using a ferric chloride-induced carotid arterial thrombosis model in mice and an *in vitro* model using rotation thromboelastometry (ROTEM) and platelet aggregometry. Infusion of aggrastat

immediately after the end of rt-PA infusion resulted in improved efficacy in preventing re-occlusion in mice treated with rt-PA alone. However, when aggrastat was administrated to mice simultaneously at the time of rt-PA bolus infusion, it did not significantly improve the thrombolytic effects. Compared with the group treated with rt-PA only, the group treated with concomitant atorvastatin and rt-PA showed improved effects in terms of shorter time to initiation of lysis or recanalization and longer persistent recanalization of the blood flow. By ROTEM assay, clot firmness at 20, 25, and 30 minutes was lower in the group treated with a combination of rt-PA and atorvastatin than in the control group. Likewise, in terms of the lysis parameter, the EX lysis index at 30 minutes was shorter in the group treated with concomitant atorvastatin and rt-PA, which implies that clot lysis was greater at 30 minutes. However, there was no significant difference in the variables of FIBTEM. These results suggest that adding atorvastatin to rt-PA produces a faster thrombolytic response through inhibition of the platelet-related pathway. Platelet aggregometry confirmed that atrovastatin inhibited platelet aggregation as maximal aggregation values and the area under curve were significantly lower in the atorvastatin-treated group. However, lipid profiles were not different between the two groups, which suggests that these effects were not due to the lipid lowering effects of atorvastatin. Our results suggest that rt-PA treatment can be improved by adjuvant use of aggrastat or atorvastatin, and that their effectiveness is due to their antiplatelet actions.

Key words: recombinant tissue plasminogen activator, thrombolysis, glycoprotein GIIb/IIIa antagonist, statin

Optimal strategy for effective dissolution of the thrombus in thrombolysis models

Young Dae Kim

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Ji Hoe Heo)

I. INTRODUCTION

Thrombolytic treatment with intravenous (IV) recombinant tissue plasminogen activator (rt-PA) is a proven therapy for acute ischemic stroke.[1] Thrombolytic treatment can lead to recanalization of an occluded artery, which is associated with favorable clinical outcome.[2] Although IV rt-PA is effective in acute

ischemic stroke, the use of IV rt-PA is limited by its narrow therapeutic time window and by the risk of intracerebral hemorrhage. Furthermore, the thrombolytic efficacy of IV rt-PA is relatively low, with a successful recanalization rate of only 20-30%,[2,3] particularly in large artery occlusion with a large thrombus burden.[4] To improve the thrombolytic efficacy, intra-arterial approaches using chemical agents or mechanical devices have been tried.[5-7] However, intra-arterial thrombolysis requires additional infrastructure including angiographic suites and experienced personnel, which limits its wide use. Recently, new thrombolytic agents have been developed, although the efficacy of these agents has not been proven through the randomized controlled trials.[8]

During thrombolytic treatment, the thrombus undergoes dynamic changes.[9,10] Our previous work revealed that lysis of the thrombus alternating with regrowth of thrombus subsequently leads to re-occlusion during or after infusion of rt-PA. In 20% of acute ischemic stroke patients, increase in thrombus volume was observed byu thin-section noncontrast computed tomography scans, which were taken before and after IV rt-PA infusion.[11] Previous reports showed that re-occlusion after successful recanalization developed in up to 30% of patients and that re-occlusion resulted in a poor outcome.[9,12,13] Platelets play a major role in the development of re-occlusion after thrombolytic treatment,[14] and

administration of glycoprotein (GP)IIb/IIIa antagonists, one of antiplatelet agents, can be beneficial in re-opening a re-occluded artery.[9] Considering the effect of GPIIb/IIIa antagonists on inhibition of platelet aggregation, combined use of GPIIb/IIIa antagonists and rt-PA might be more effective for suppression of thrombus re-growth compared with infusion of rt-PA alone. However, there is a little data regarding the potential benefits of combining rt-PA and GPIIb/IIIa inhibitors in terms of the efficacy of recanalization.

Thrombolytic efficacy is determined by the patient's underlying characteristics, vascular status, and concomitant medication.[15-17] Statins, which are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)-reductase inhibitors, block the synthesis of mevalonate and inhibit the biosynthesis of cholesterol. Their efficacy in preventing cardiovascular disease has been established in many clinical studies.[18] However, it has been reported that statins also have many other beneficial actions, known as pleiotropic effects, on endothelial cells and platelets.[19,20] These pleiotropic effects are observed shortly after statin treatment.[21] A recent report showed that administration of statin within 72 hours of stroke onset was associated with both early and late favorable outcomes in ischemic stroke patients.[22] Combination treatment with rt-PA and atrovastatin, thrombolytic efficacy may potentiate the thrombolytic efficacy in animal stroke

models, as combination treatment blocks the expansion of the ischemic lesion and improves the neurologic outcome and microvascular patency.[21,23] These benefits may be due to the pleiotropic effects of statins, including reduction of platelet activation and an increase in fibrinolysis. When considering this evidence, statins may enhance the thrombolytic efficacy of rt-PA. However, there has been little data studying the effects of statin on recanalization.

The aim of this study was to determine whether the combination of IV rt-PA with a GPIIb/IIIa antagonist or atorvastatin would enhance thrombolytic efficacy.

II. MATERIALS AND METHODS

The care and use of laboratory animals in this experiment were performed according to institutionally approved protocols in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

1. *In vivo* model for investigation of combined thrombolytic agents

A. Animal model and $FeCl_3$-induced carotid artery thrombosis

Eight-week-old male mice from the Institute for Cancer Research (ICR) were used in these experiments. For operative procedures, animals were anesthetized by inhalation of 5% isoflurane in a mixture of 70% N_2O and 30% O_2. Anesthesia was maintained with 2% isoflurane. During the operative procedures, body temperature was monitored using a rectal temperature probe, and kept at 37.0 ± 0.2 ℃ using a homeothermic blanket control unit and a heating pad (Harvard Apparatus, Holliston, MA, USA). A $FeCl_3$-induced carotid thrombosis model was used to test the thrombolytic activity of thrombolytic agents in vivo. After shaving and sterile preparation of the mouse, the left common carotid artery was carefully dissected under a surgical microscope after a midline incision of the neck. An ultrasonic Doppler flow

probe (MA0.7PSB; Transonic Instruments, Ithaca, NY, USA) was placed around the mid-portion of the common carotid artery (CCA). Carotid blood flow was monitored with a Transonic TS420 Blood Flow Meter (Transonic Instruments, Ithaca, NY, USA) and blood flow data was obtained using an iWorx IX-304T data acquisition system (iWorx Systems, Inc., Dover, NH). Baseline flow of the CCA was measured for five minutes before the ultrasonic Doppler flow probe was removed. After drying the surface of the CCA by removing tissue fluid from the surface of the CCA and around the CCA, thrombosis was induced by placing a filter paper (700 μm × 500 μm) saturated with 50% $FeCl_3$ on the adventitial surface of the midpoint of the exposed CCA for five minutes. After removing the filter paper, the CCA was washed with normal saline and its blood flow was recorded by placing the ultrasonic Doppler flow probe. Thrombus formation and arterial occlusion were determined by a decrease in blood flow, and complete occlusion was defined as a sustained absence of blood flow for ten minutes.

B. Intravenous thrombolysis *in vivo* model

Ten minutes after occlusion of the CCA, thrombolytic agents were administrated intravenously via the left femoral vein using an infusion pump (KDS100 syringe pump; KD Scientific, Holliston, MA, USA) connected to PE-

10 tubing. Carotid blood flow was continuously monitored for two hours from the initial time of drug administration.

(1) rt-PA infusion used in mice model

The total dose of rt-PA (Actilyse®; Boehringer Ingelheim, Germany) for IV administration was 9 mg/kg, which is 10 times higher than a dose in humans, as the plasma level of plasminogen activator inhibitor-1 is elevated in rodents and the specific fibrinolytic effect of rt-PA is at least 10 times lower in rodents than in humans.[24] Each mouse received rt-PA intravenously with 10% of total volume as an initial bolus, followed by infusion of the remainder (90%) over 1 hour.

(2) Aggrastat infusion in the mouse model

We investigated the dose-response of aggrastat (Tirofiban®; Merck&Co USA) because little data is available regarding the optimum dose of aggrastat in a mouse model. Animals were randomly divided into seven groups (five mice in each group): 0 (normal saline), 0.5, 1.25, 2.5, 3.75, 5, and 10 mg/kg of aggrastat administration groups. Ten percent of the dose was initially administered as an intravenous bolus, and the rest was infused continuously for 60 min.

The average blood flow was calculated in each animal according to the dose

of aggrastat. The thrombolytic effect was calculated as described below and expressed as a percent of mean blood flow at baseline; [mean blood flow during the two hours after administration of aggrastat / mean baseline blood flow) × 100 (%)]. The mean values of each group in the dose-response study were calculated and graphed on a standard thrombolytic activity curve (mean ± SD).

(3) Atorvastatin use in the mouse model

Atorvastatin (Lipitor®; Pfizer, Seoul, Korea) was administrated intraperitoneally at a dose of 10 mg/kg immediately after confirmation of thrombus formation at the left CCA by the blood flow meter. The dose of atorvastatin (10 mg/kg) was selected based on results published previously,[19,25] as the amount showing upregulation of eNOS in thrombocytes and decreased platelet activation or cerebral ischemia.

(4) Sequential administration of agents in an in vitro model

For investigation of beneficial effect of sequentially administrated regimens, three scenarios were tested: (1) IV aggrastat after the end of IV rt-PA infusion, (2) combination treatment with IV rt-PA and IV aggrastat, and (3) combination treatment with IV rt-PA and IP atorvastatin. The time to initiate lysis of the thrombus and for recanalization, duration of persistent recanalization, the

presence of re-occlusion, and final lysis of the thrombus were compared among the three groups.

C. Assessment of recanalization

The presence of recanalization was assessed by continuous measurement of blood flow. Baseline and continuous blood flow data after CCA occlusion were acquired using iWorx Labscribe 2 data acquisition software (version 2.045000). The raw blood flow per minute data of all mice included in each group were merged into one graph, which was used in the analysis of time to recanalization, the presence of re-occlusion, and final lysis of the thrombus.

(1) Onset time to initial lysis of thrombus

Time from administration of thrombolytic agents to initiation of lysis was investigated. The definition of initiation of lysis was a blood flow of at least ≥ 30% of the baseline level as documented using a Transonic TS420 Blood Flow Meter.

(2) Time to persistent recanalization

Time from administration of thrombolytic agents to persistent recanalization was assessed. Persistent recanalization was defined as the restoration of blood

flow to at least ≥ 50% of the baseline level maintained for longer than 30 minutes.

(3) The presence of re-occlusion

Re-occlusion after successful recanalization was defined when the blood flow, which was restored to ≥50% of the baseline level, decreased to below 50% of the baseline level.

(4) Total duration of recanalization

The definition of total duration of recanalization in this study was the summation of the time from recanalization with restoration of blood flow to at least ≥ 50% of the baseline level to time of re-occlusion.

(5) Final lysis of thrombus

After two hours of infusion with thrombolytic agents, final mean blood flow was checked. In case where final blood flow was ≥ 100%, blood flow was considered to be 100%.

2. *In vitro* model for investigation of statin effect on thrombolysis

A. Exposure of mice to atorvastatin

Mice were treated with intraperitoneal injection of atorvastatin at a dose of 10 mg/kg, and were sacrificed 60 min after the end administration of atorvastatin. The control group received normal saline. Mice were anesthetized with isoflurane and blood (900 μl) to be used for thromboelastography or platelet aggregometry was drawn by cardiac puncture.

B. Rotation Thromboelastometry

For the investigation of possible mechanism of enhanced thrombolytic efficacy in mice that were simultaneously treated with atorvastatin and rt-PA, we used Rotation Thrombolelastometry (ROTEM).

Whole blood samples were analyzed using the protocol recommended by the manufacturer. ROTEM measures the viscoelastic properties of the clot and provides information on the speed of coagulation initiation, the kinetics of clot growth, clot strength and lysis (Figure 1). Analyses were performed by pipetting 300 μL citrated whole blood and 20 μL 0.2 M calcium chloride with specific activators into a plastic cup as previously described.[26] The following tests were performed: EXTEM, which uses rabbit brain thromboplastin as the activator, and FIBTEM, which assesses the fibrin-based clot using cytochalasin-D to inhibit the contribution of platelets. Tests were simultaneously done at 37℃ in four channels. Sample were tested against atorvastatin only (n=5), combined

atorvastatin and rt-PA (n=5) or saline (n=5). The concentration of rt-PA used was 0.4 μg and rt-PA was directly put into the working cup. Characteristics of the clots obtained by ROTEM are presented in Table 1.

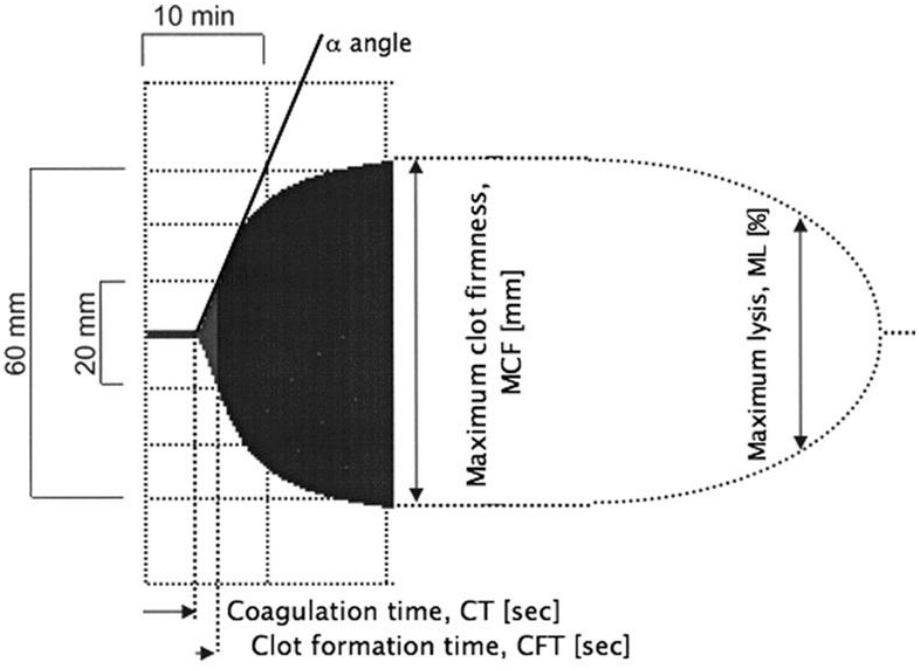

Figure 1. Normal thromboelastography in Rotation Thrombolelastometry (ROTEM) tracing.

Table 1. Parameters used for ROTEM assay.

Variables	Parameter	Definition	Unit
Coagulation activation and clot polymerization parameters			
CT	Coagulation time	Time from the test start to an amplitude of 2 mm	s
CFT	Clot formation time	Time between 2 mm amplitude and 20 mm amplitude	s
A	α-angle	Angle between the baseline and a tangent to the clotting curve through the 2-mm point	degree
Clot firmness parameters			
MCF	Maximum clot firmness	Maximum amplitude reached during the test	mm
A(x)	Amplitude (firmness) at time x	Clot firmness (in mm amplitude) at the respective time point after CT	mm
Clot lysis parameters			
ML	Maximum lysis	Maximum lysis detected during the run time	%
LI(x)	Lysis index at time x minutes	Ratio of the amplitude and MCF at a given time point after CT	%
Research parameters			
maxV	Maximum velocity	Maximum of the 1st derivative of the curve	mm/min
maxV-T	Time to maximum velocity	Time from reaction start to the maximum of the 1st derivative of the curve	s
AUC	Area under 1st derivative curve	Area under 1st derivative curve from start of the derivative curve until MCF is reached	mm X 100
CFR	Clot formation rate	Angle between the baseline and the tangent at the maximum slope	degree
ACF	Actual clot firmness or last clot firmness	Clot firmness at the actual time point of observation	mm
MCF-T	MCF-time	Time from CT until MCF is reached	s
MCE	Maximum clot elasticity	MCE=100 X MCF/(100-MCF)	

AR(x)	Area under the curve at time x	Area under the curve from CT to the respective time point (minutes)	mm^2
LOT	Lysis onset time	Time span from CT to the start of significant lysis	s
CLR	Clot lysis rate	Strongest lysis described by the angle between the baseline and the tangent to the declining firmness curve at the minimum of the 1st derivative	degree
LT	Lysis time	Time from CT until clot firmness decreased to 10% of the MCF	s
G	G=5000MCF/(100-MCF)	Shear elastic modulus strength	

C. Platelet aggregometry

The inhibitory effects of atorvastatin on platelet aggregation were investigated using platelet aggregometry (Chronolog 700; Chronolog Corporation, Havertown, PA, USA). Sixty minutes after intraperitoneal administration of atorvastatin at a dose of 10 mg/kg, blood (900 μl) was drawn by cardiac puncture from four mice anesthetized with isoflurane. The blood was drawn into a syringe containing 100 μl of 150 USP sodium heparin solutions, resulting in a final heparin concentration of 15 USP/ml. A total of 500 μl of heparinized whole blood was mixed with the same volume of normal saline. The same volume of normal saline was added as a control. A solution of 20 μM ADP (Chronolog Corporation, Havertown, PA, USA) were used as a platelet activator. Platelet aggregation activity was measured with an impedance method in a platelet aggregometer. The effect of platelet disaggregation was calculated as a percentage of restoration to the baseline level.

D. Statistical analysis

All statistical computations were performed using SPSS (version 18.0, SPSS Inc, Chicago, IL. USA). The categorical variables were compared using a chi square test or Fisher's exact test and continuous variables were compared using Mann-Whitney test, as appropriate. The values are presented as the mean ±

standard deviation (SD). $P < 0.05$ was considered statistically significant.

III. RESULTS

1. Complete occlusion according to the concentration of FeCl3

For the assessment of recanalization after drug treatment, blood flow should be monitored before and for sometime after the end of drug administration. The arterial occlusive state should be sustained during that time. We investigated which concentration of $FeCl_3$ was appropriate for leading to complete occlusion in our study. As the concentration of $FeCl_3$ increased, blood flow at the carotid artery reduced and time to occlusion decreased. A concentration of 50% $FeCl_3$ was chosen for the remainder of the experiments, as the carotid artery was in a completely occluded state without spontaneous recanalization for a 2-hour period in all tested mice.

2. Dose dependent thrombolytic efficacy of aggrastat

Infusion of aggrastat resulted in the restoration of blood flow in a dose-dependent manner. Blood flow was restored to nearly baseline levels at a dose of 10 mg/kg (Figure 2). In each mouse, blood flow did not recover gradually, but instead fluctuated, indicating that re-occlusion occurs frequently during the process of recanalization (Figure 2).

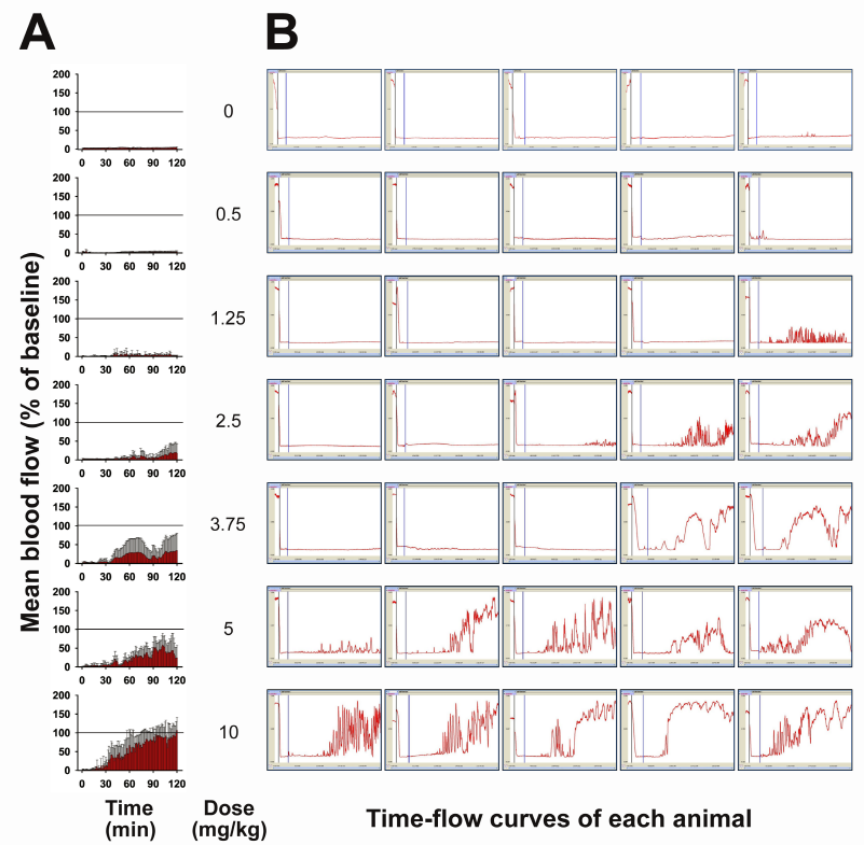

Figure 2. Dose-dependent responses of aggrastat. The Transonic TS420 Blood Flow Meter showed that the thrombolytic efficacy of aggrastat gradually increased as the dose increased. During recanalization, fluctuating blood flow was observed. **A:** Time-patterns of blood flow according to concentration of aggrastat. **B:** Time-flow curves of all mice in each group.

3. Combined thrombolytic effects of two agents

A. Combination treatment of rt-PA and aggrastat

When rt-PA and aggrastat were given to mice simultaneously, thrombolytic effects were not enhanced although the time to recanalization seemed to be slightly shorter in comparison with those treated with rt-PA alone (18.4 ± 13.3 minute in simultaneous administration of rt-PA and aggrast vs. 32.1 ± 19.9 minute in rt-PA alone, p=0.347). However, infusion of aggrastat immediately after the end of rt-PA infusion resulted in improved efficacy, in that re-occlusion, which was seen in mice treated with rt-PA alone, was effectively prevented (Figure 3). The final lysis of thrombus was significantly different between the rt-PA alone group and the sequential administration group (28.0 ± 46.4 % vs. 124.8 ± 9.8 %, p=0.009).

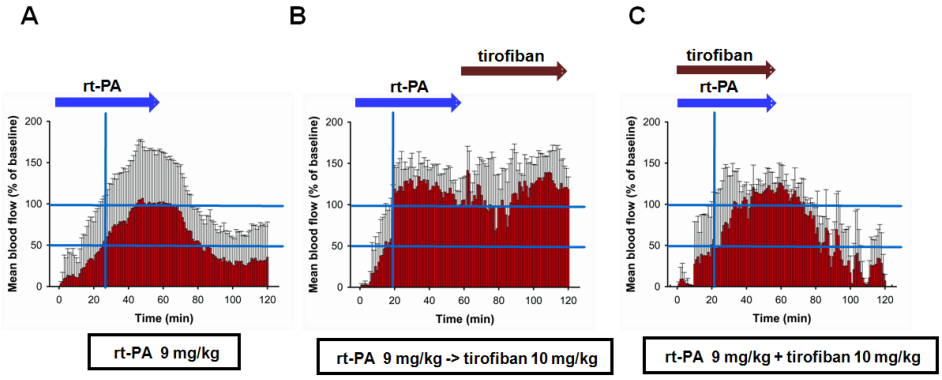

Figure 3. Thrombolytic efficacy according to administration methods of aggrastat. Sequential administration of aggrastat effectively prevented re-occlusion. **A:** rt-PA only. **B:** rt-PA followed by aggrastat. **C:** Simultaneous administration of rt-PA and aggrastat.

B. Combination treatment with rt-PA and atorvastatin

Compared with the rt-PA group, concomitant use of atorvastatin and rt-PA showed improved effects in terms of a shorter time to initiation of lysis or recanalization and longer persistent recanalization of blood flow (Figure 4), although these differences were not statistically significant (Table 2). Notably, the response to rt-PA was less variable in the group treated with simultaneous use of rt-PA and atorvastatin (Figure 5, vertical arrow).

None of the mice experienced bleeding complications such as intracerebral hemorrhage, intraperitoneal hematoma, or airway hemorrhage.

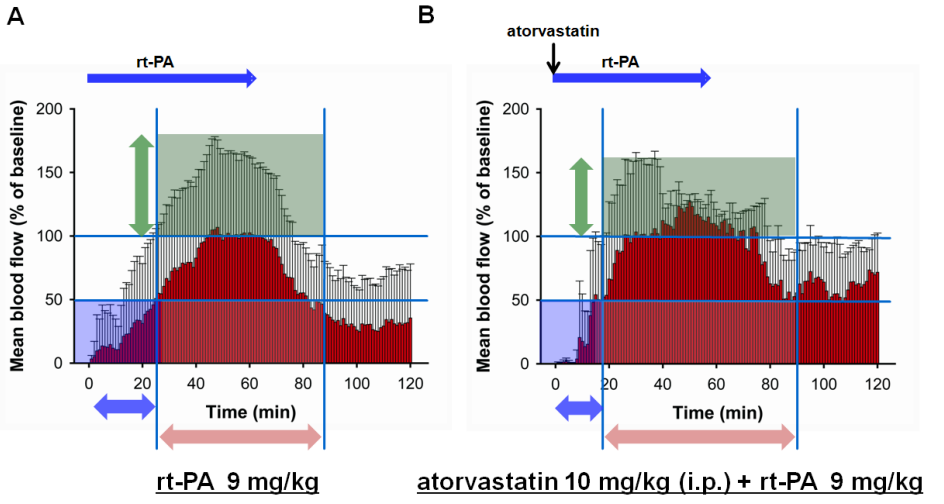

Figure 4. Thrombolytic efficacy of rt-PA and atorvastatin. Simultaneous administration of rt-PA and atorvastatin showed earlier recanalization (blue arrow) and longer maintenance of effective blood flow (red arrow). Effective blood flow was defined as the restored blood flow above 50% of the baseline. **A:** rt-PA only. **B:** Simultaneous administration of rt-PA and atorvastatin.

Table 2. Effect of atorvastatin on thrombolysis with rt-PA.

	rt-PA	rt-PA + atorvastatin	p
Onset time to initial lysis of thrombus, minute	32.1 ± 19.9	18.7 ± 12.5	0.175
Time to persistent recanalization, minute	27.8 ± 20.1	18.8 ± 12.5	0.347
Total duration of recanalization, minute	51.8 ± 18.2	87.3 ± 15.6	0.028
Final lysis of thrombus, %	28.0 ± 46.4	70.4 ± 26.0	0.115

Data are given as mean ± standard deviation.

4. Effects of atorvastatin on rt-PA induced thrombolysis

A. Combination treatment with atorvastatin and rt-PA

The effect and possible mechanism of atorvastatin on clot lysis were investigated with the use of a ROTEM analyzer. A dose of 0.4 μg rt-PA was used to induce clot lysis since this concentration showed complete lysis in ROTEM (Figure 5).

The percentage of rt-PA-induced clot lysis increased in mice treated with atorvastatin (Figure 6). Among the variables, EX A (20), EX A (25), and EX A (30) were smaller in the group treated with a combination of rt-PA and atorvastatin (Table 3) than those in the control group. Likewise, in the lysis parameters, EX LI (30) was shorter in the combination group, which implies

that clot lysis was greater at 30 minutes. However, there were no significant differences in the variables of FIBTEM (Table 4). These results suggest that the addition of atorvastatin to rt-PA produces a faster thrombolytic response through inhibition of platelet-related pathway.

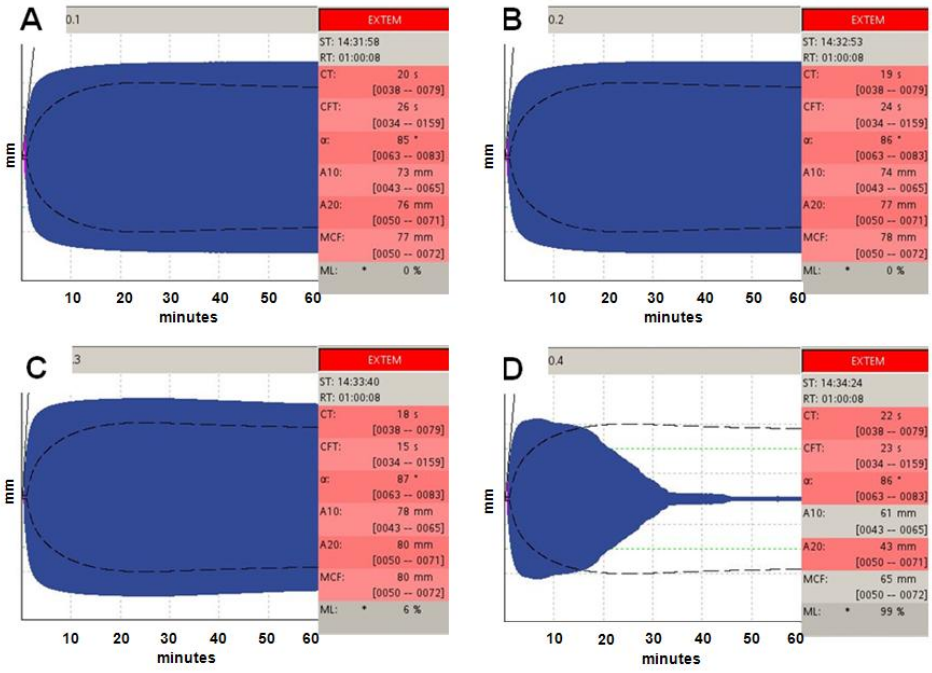

Figure 5. Thromboelastometry analyses (EXTEM) according to concentration of rt-PA. **A:** 0.1 µg rt-PA. **B:** 0.2 µg rt-PA. **C:** 0.3 µg rt-PA. **D:** 0.4 µg rt-PA.

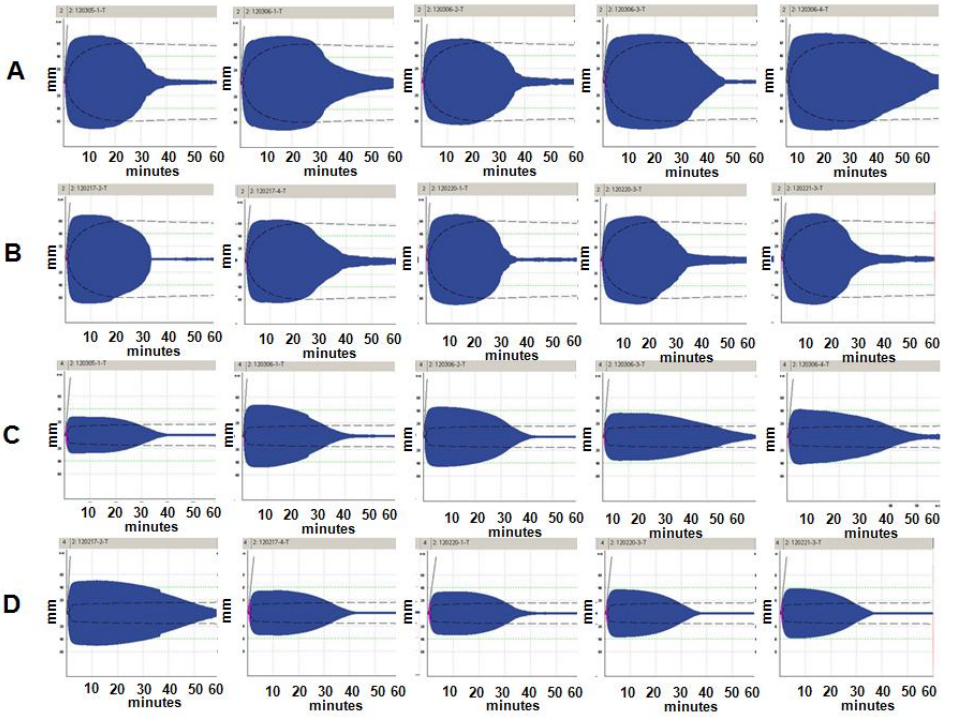

Figure 6. Thromboelastometry analyses (EXTEM) of combination treatment with rt-PA and atorvastatin. EXTEM **A, B** (A: control with rt-PA. B: atorvastatin with rt-PA). FIBTEM **C, D** (B: control with rt-PA. D: atorvastatin with rt-PA).

Table 3. Comparison of EXTEM variables between groups according to combination treatment of rt-PA and atorvastatin.

	rt-PA	rt-PA + atorvastatin	p
EX_CT, second	22.4 ± 0.6	21.6 ± 1.7	0.313
EX_CFT, second	17.2 ± 3.4	20.6 ± 3.4	0.073
EX_α, °	86.8 ± 0.5	86.2 ± 0.8	0.189
EX_A5, mm	67.4 ± 3.9	66.6 ± 2.1	0.396
EX_A10, mm	71.2 ± 3.0	69.2 ± 2.2	0.089
EX_A15, mm	72.0 ± 3.0	69.0 ± 2.0	0.074
EX_A20, mm	70.4 ± 3.7	62.8 ± 1.9	0.011
EX_A25, mm	65.2 ± 7.6	50.4 ± 3.8	0.009
EX_A30, mm	53.2 ± 12.4	28 ± 8.9	0.016
EX_MCF, mm	72.0 ± 3.5	69.6 ± 2.3	0.090
MX_ML, %	92.8 ± 5.2	96.4 ± 2.6	0.344
EX_LI30, %	73.6 ± 15.7	40.4 ± 13.7	0.027
EX_LI45, %	18.8 ± 17.0	7.0 ± 3.9	0.142
EX_LI60, %	NA	NA	
EX_maxV, mm/min	75.2 ± 11.1	61.4 ± 10.2	0.047
EX_maxV-T, second	27.4 ± 0.6	27.0 ± 1.2	0.729
EX_AUC, mm x 100	6950.8 ± 287.1	6749.2 ± 183.2	0.076

EX_MCE	261.8 ± 37.8	230.4 ± 21.7	0.076
EX_MCF-T, second	796.2 ± 7.0	669.6 ± 101.7	0.076
EX_CFR, °	86.8 ± 0.5	86.2 ± 0.8	0.189
EX_LT, second	2778.5 ± 551.2	2317.4 ± 388.7	0.142
EX_CLR, °	73.5 ± 2.7	71.0 ± 7.5	0.386
EX_ACF, mm	5.4 ± 3.7	3.2 ± 1.8	0.343
EX_G	13100.4 ± 1888.1	11525.2 ± 1077.4	0.076
EX_AR5, mm^2	553.6 ± 36.3	541.6 ± 26.8	0.347
EX_AR10, mm^2	1251 ± 70.3	1223.4 ± 46.3	0.251

Data are given as mean ± standard deviation. Abbreviations are defined as follows: EX, EXTEM; CT, Coagulation time; CFT, Clot formation time; α, α-angle; A(x), Amplitude at time x minutes; MCF, Maximum clot firmness; ML, Maximum lysis; LI(x), Lysis index at time x minutes; NA, not accessible; maxV, Maximum velocity; maxV-T, Time to maximum velocity; AUC, Area under 1^{st} derivative curve; MCE, Maximum clot elasticity; MCF-T, MCF-time; CFR, Clot formation rate; LT, Lysis time; CLR, Clot lysis rate; ACF, Actual clot firmness; G, G=5000MCF/(100-MCF); AR(x), Area under the curve at time x minutes.

Table 4. Comparison of FIBTEM variables between groups according to combination treatment of rt-PA and atorvastatin.

	rt-PA	rt-PA + atorvastatin	p
FIB_CT, second	23.4 ± 0.9	23.4 ± 1.3	0.910
FIB_CFT, second	25.6 ± 11.3	35.4 ± 13.4	0.295
FIB_α, °	86.2 ± 0.8	85.0 ± 1.9.0	0.167
FIB_A5, mm	40.8 ± 7.9	39.4 ± 6.4	0.754
FIB_A10, mm	40.0 ± 8.0	39.2 ± 6.9	0.675
FIB_A15, mm	38.6 ± 6.9	38.4 ± 7.1	0.600
FIB_A20, mm	35.6 ± 6.4	35.0 ± 7.8	0.492
FIB_A25, mm	31.2 ± 5.8	29.8 ± 9.2	0.459
FIB_A30, mm	23.8 ± 6.6	21.4 ± 12.4	0.343
FIB_MCF, mm	40.8 ± 7.9	39.6 ± 6.8	0.754
MX_ML, %	96.8 ± 3.1	97.8 ± 4.4	0.331
FIB_LI30, %	58.8 ± 14.8	52.2 ± 20.0	0.530
FIB_LI45, %	13.6 ± 15.8	10.0 ± 18.0	0.396
FIB_LI60, %	NA	NA	
FIB_maxV, mm/min	66.4 ± 18.3	52.0 ± 24.2	0.142
FIB_maxV-T, second	29.2 ± 1.6	28.6 ± 1.3	0.513
FIB_AUC, mm x 100	3840.2 ± 803.6	3818.2 ± 630.6	0.754

FIB_MCE	70.8 ± 21.7	67.6 ± 20.9	0.754
FIB_MCF-T, second	298.0 ± 61	483.2 ± 190.2	0.175
FIB_CFR, °	86.4 ± 1.1	85.2 ± 1.8	0.159
FIB_LT, second	2689.4 ± 522.2	2217.8 ± 135.9	0.142
FIB_CLR, °	50.3 ± 21.4	42.4 ± 19.5	0.177
FIB_ACF, mm	1.4 ± 1.1	1.4 ± 2.1	0.585
FIB_G	3543.8 ± 1094.6	3375.0 ± 1044.0	0.754
FIB_AR5, mm^2	353.2 ± 69.0	333.0 ± 63.4	0.754
FIB_AR10, mm^2	757.0 ± 150.3	667.2 ± 40.4	0.249

Data are given as mean ± standard deviation. Abbreviations are defined as follows: FIB, FIBTEM; CT, Coagulation time; CFT, Clot formation time; α, α-angle; A(x), Amplitude at time x minutes; MCF, Maximum clot firmness; ML, Maximum lysis; LI(x), Lysis index at time x minutes; NA, not accessible; maxV, Maximum velocity; maxV-T, Time to maximum velocity; AUC, Area under 1^{st} derivative curve; MCE, Maximum clot elasticity; MCF-T, MCF-time; CFR, Clot formation rate; LT, Lysis time; CLR, Clot lysis rate; ACF, Actual clot firmness; G, G=5000MCF/(100-MCF); AR(x), Area under the curve at time x minutes.

B. Combination treatment with atorvastatin but without rt-PA

In contrast with the group with combined rt-PA and atorvastatin treatment, the group treated with atorvastatin alone did not show any thrombolytic effects (Figure 7). When the variables including EXTEM or FIBTEM on ROTEM were compared between the two groups, there were no differences except in FIB CT and FIB maxV-T (Table 5 and 6).

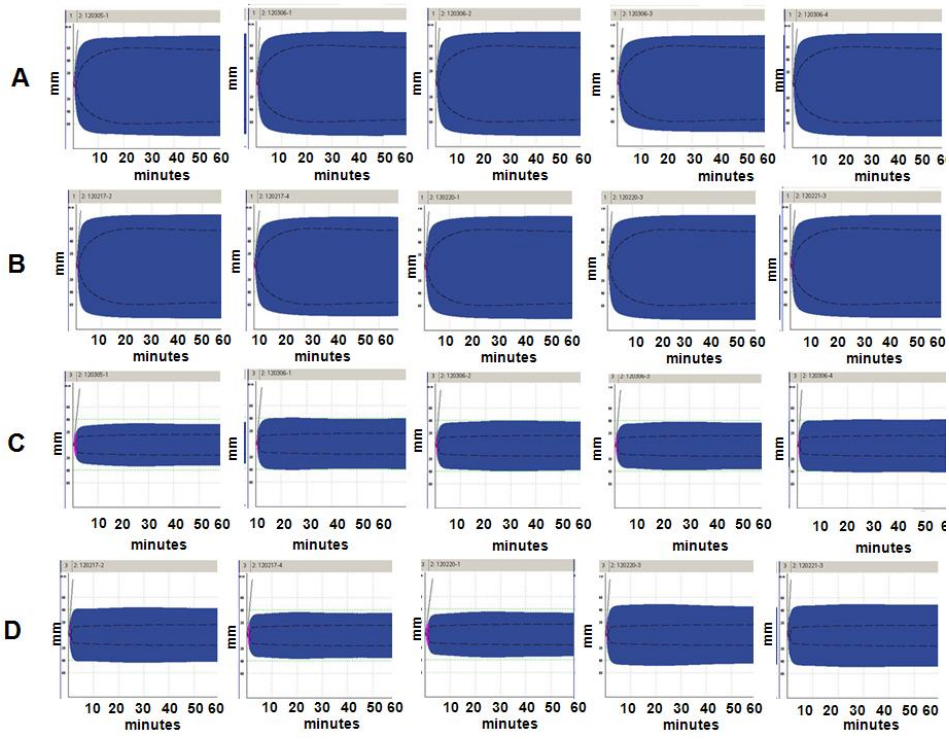

Figure 7. Thromboelastometry analyses (EXTEM) of acutely administrated atorvastatin. EXTEM **A, B** (A: atorvastatin B: control). FIBTEM **C, D** (C: atorvastatin. D: control).

Table 5. Comparison of EXTEM variables between groups with or without atrovastatin.

	control	Atrovastatin	p
EX_CT, second	20.4 ± 1.5	19.2 ± 1.1	0.146
EX_CFT, second	17.2 ± 3.7	19.8 ± 3.7	0.245
EX_α, °	87.0 ± 0.7	86.4 ± 0.5	0.166
EX_A5, mm	71.4 ± 2.7	72.4 ± 2.3	0.525
EX_A10, mm	76.2 ± 2.5	77.2 ± 2.0	0.456
EX_A15, mm	78.0 ± 2.3.0	79.0 ± 1.6	0.525
EX_A20, mm	79.2 ± 2.5	80.2 ± 1.5	0.665
EX_A25, mm	79.6 ± 2.5	80.8 ± 1.9	0.456
EX_A30, mm	80.4 ± 2.3	81.2 ± 1.5	0.745
EX_MCF, mm	80.4 ± 2.3	81.2 ± 1.5	0.745
MX_ML, %	0.2 ± 0.4	0.0 ± 0.0	0.317
EX_LI30, %	100.0 ± 0.0	100.0 ± 0.0	1.00
EX_LI45, %	100.0 ± 0.0	100.0 ± 0.0	1.00
EX_LI60, %	NA	NA	
EX_maxV, mm/min	76.8 ± 14.4	66.4 ± 10.8	0.173
EX_maxV-T, second	27.2 ± 1.3	26.0 ± 1.9	0.309
EX_AUC, mm x 100	8059.0 ± 578.6	7943.2 ± 135.0	0.602

EX_MCE	417.6 ± 55.6	438.2 ± 46.8	0.917
EX_MCF-T, second	2199.2 ± 285.3	2007.2 ± 284.9	0.251
EX_CFR, °	87.0 ± 0.7	86.6 ± 0.5	0.339
EX_LT, second			
EX_CLR, °	20.3 ± 28.3	3.0 ± 0	0.078
EX_ACF, mm	80.6 ± 2.3	81.2 ± 1.5	0.750
EX_G	20882.4 ± 2790.2	21920.4 ± 2356.3	0.917
EX_AR5, mm^2	573.6 ± 29.0	578.0 ± 27.9	0.675
EX_AR10, mm^2	1319.2 ± 53.5	1331.2 ± 48.3	0.602

Data are given as mean ± standard deviation. Abbreviations are defined as follows: EX, EXTEM; CT, Coagulation time; CFT, Clot formation time; α, α-angle; A(x), Amplitude at time x minutes; MCF, Maximum clot firmness; ML, Maximum lysis; LI(x), Lysis index at time x minutes; NA, not accessible; maxV, Maximum velocity; maxV-T, Time to maximum velocity; AUC, Area under 1^{st} derivative curve; MCE, Maximum clot elasticity; MCF-T, MCF-time; CFR, Clot formation rate; LT, Lysis time; CLR, Clot lysis rate; ACF, Actual clot firmness; G, G=5000MCF/(100-MCF); AR(x), Area under the curve at time x minutes.

Table 6. Comparison of FIBTEM variables between groups with or without atorvastatin.

	control	Atrovastatin	p
FIB_CT, second	23.0 ± 1.7	20.2 ± 1.1	0.029
FIB_CFT, second	36.8 ± 11.6	30.0 ± 18.5	0.465
FIB_α, °	85.4 ± 0.5	86.0 ± 2.0	0.395
FIB_A5, mm	36.6 ± 4.1	40.4 ± 7.1	0.344
FIB_A10, mm	37.6 ± 3.5	41.6 ± 6.5	0.346
FIB_A15, mm	38.4 ± 3.3	42.6 ± 6.5	0.344
FIB_A20, mm	38.8 ± 3.1	43.2 ± 6.5	0.347
FIB_A25, mm	39.0 ± 3.0	43.2 ± 6.5	0.346
FIB_A30, mm	39.0 ± 3.0	43.0 ± 6.8	0.344
FIB_MCF, mm	39.2 ± 3.1	42.8 ± 6.5	0.402
MX_ML, %	3.6 ± 1.5	4.0 ± 2.4	0.519
FIB_LI30, %	99.2 ± 1.8	99.2 ± 1.3	0.700
FIB_LI45, %	97.2 ± 1.3	97.0 ± 2.3	0.670
FIB_LI60, %	NA	NA	
FIB_maxV, mm/min	52.6 ± 5.7	66.4 ± 28.1	0.530
FIB_maxV-T, second	28.0 ± 0.0	25.6 ± 1.3	0.014
FIB_AUC, mm x 100	3781.6 ± 287.2	4144.0 ± 631.8	0.465

FIB_MCE	64.8 ± 7.9	77.2 ± 20.4	0.347
FIB_MCF-T, second	1292.8 ± 353.5	1053.6 ± 503.9	0.347
FIB_CFR, °	85.4 ± 0.5	86.2 ± 1.6	0.386
FIB_LT, second	NA	NA	
FIB_CLR, °	6.5 ± 0.7		0.221
FIB_ACF, mm	38.2 ± 3.4	41.8 ± 6.1	0.347
FIB_G	3235.8 ± 396.5	3860.8 ± 1014.9	0.347
FIB_AR5, mm^2	307.2 ± 35.7	348.8 ± 66.2	0.347
FIB_AR10, mm^2	678.0 ± 73.2	760.6 ± 135.7	0.347

Data are given as mean ± standard deviation. Abbreviations are defined as follows: FIB, FIBTEM; CT, Coagulation time; CFT, Clot formation time; α, α-angle; A(x), Amplitude at time x minutes; MCF, Maximum clot firmness; ML, Maximum lysis; LI(x), Lysis index at time x minutes; NA, not accessible; maxV, Maximum velocity; maxV-T, Time to maximum velocity; AUC, Area under 1^{st} derivative curve; MCE, Maximum clot elasticity; MCF-T, MCF-time; CFR, Clot formation rate; LT, Lysis time; CLR, Clot lysis rate; ACF, Actual clot firmness; G, G=5000MCF/(100-MCF); AR(x), Area under the curve at time x minutes.

C. Effects of atorvastatin on platelet aggregation

The early effect of atorvastatin on platelet aggregation was examined using blood obtained from mice treated with atorvastatin (10 mg/kg, IP) one hour before blood sampling. Platelet aggregometry showed that atrovastatin inhibited platelet aggregation (Figure 8). Maximal aggregation values inhibited by atorvastatin were compared with a control value of maximal aggregation (saline treated group). Although the inhibitory effect of atorvastatin varied, an overall effect of platelet aggregation by atorvastatin was observed. Maximal aggregation values and the area under curve were significantly lower in the atorvastatin-treated group ($p<0.05$) (Table 7), which suggests that acute atorvastatin therapy may inhibit platelet aggregation.

However, the lipid profiles were not different between the two groups, and as a result, these effects are not attributable to the lipid lowering effects of atorvastatin (Table 8).

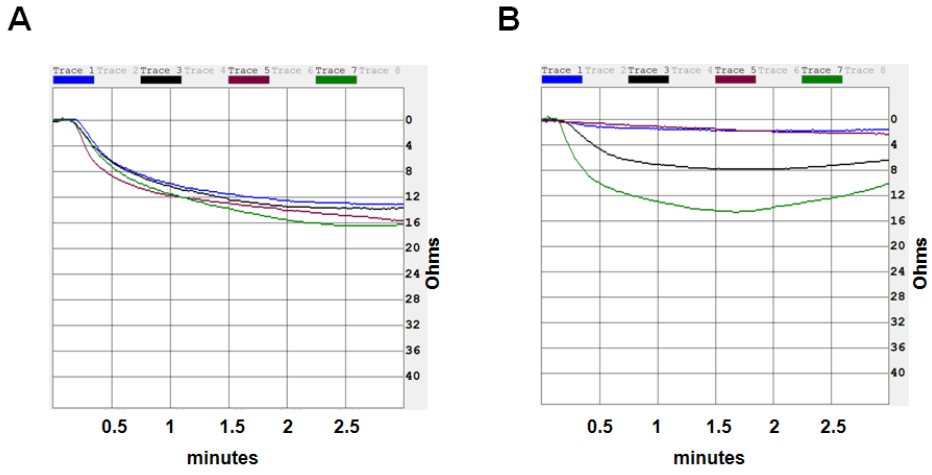

Figure 8. Results of platelet aggregometry showing inhibition of platelet aggregation. **A:** control. **B:** atorvastatin.

Table 7. Comparison of platelet aggregometry variables between groups.

	control	atorvastatin	p
Maximum amplitude, ohms	15.5 ± 1.7	6.8 ± 6.2	0.034
Slope, degree	24.0 ± 3.7	13.5 ± 14.2	0.203
Lag time, minute	5.3 ± 1.5	16.5 ± 15.2	0.191
AUC, mm x 100	60.6 ± 8.3	22.8 ± 17.6	0.008

Table 8. Comparison of lipid profiles between groups.

	control	Atorvastatin	p
Total cholesterol, mg/dL	147.8 ± 36.9	157.8 ± 5.1	0.248
Triglyceride, mg/dL	70.0 ± 37.7	119.0 ± 55.7	0.149
High density lipoprotein, mg/dL	79.5 ± 17.1	82.8 ± 10.5	0.564
Low density lipoprotein, mg/dL	5.8 ± 1.3	8.5 ± 2.4	0.144

IV. DISCUSSION

In this study, we demonstrated that the concomitant use of aggrastat with rt-PA can improve thrombolytic efficacy and this beneficial effect is greater when aggrastat is sequentially administrated after rt-PA infusion. Simultaneous administration of atorvastatin and rt-PA may lead to improved thrombolytic effect. These effects were ascertained by the inhibition of the platelet-related pathway.

The clinical usefulness of a GPIIb/IIIa inhibitor in acute ischemic stroke is controversial. A clinical trial using Abxicimab in acute ischemic stroke within 5 hours of stroke onset failed to demonstrate either safety or efficacy and was terminated prematurely.[27] However, another highly selective GPIIb/IIIa antagonist, aggrastat which is a fast-acting nonpeptide GPIIb/IIIa platelet receptor antagonist, showed beneficial effects in ischemic stroke patients. Recently, the Safety of Tirofiban in acute Ischemic Stroke (SaTIS) trial demonstrated that the use of aggrastat in acute ischemic stroke is safe within a wider time window after symptom onset as the risk of intracranial hemorrhage was not higher than that of placebo and had potential benefit in mortality reduction (2.3% vs. 8.7%, p=0.03).[28] During rt-PA infusion, the thrombus,

which occludes the artery, undergoes dynamic changes. In this study, lysis of the thrombus and rethrombosis occurred during rt-PA infusion. Thrombolytic agents paradoxically result in thrombogenic condition within the first hours after treatment by increasing platelet activation and thrombin activity. These conditions can persist for up to 3 days,[9,12,13,29,30] but can be reversed with antiplatelet agents such as GPIIb/IIIa inhibitors, which may result in potentiation of thrombolytic efficacy. In patients receiving IV rt-PA treatment, combined use of aggrastat showed prevention of re-occlusion, leading to enhancement of the recanalization rate,[31] which is reflected in our results. Considering the dynamic change of the thrombus during rt-PA treatment and the safety of aggrastat in acute ischemic stroke, adjuvant administration of aggrastat with rt-PA may be considered for improving thrombolytic effects.

Our results demonstrated that the efficacy of adjuvant administration of aggrastat may be dependent on the timing of infusion. During rt-PA infusion, successful recanalization was achieved in 25 % patients within the first 30 minutes, 50% within 30-60 minutes, 11% within 61-120 minutes, and 14% after the first 2 hours.[32] As discussed above, thrombolytic treatment with rt-PA can activate platelets and induce massive hypercoagulable state within the first hour after treatment that can persist for up to 3 days.[29] In residual thrombus induced

by thrombolytic agents, platelets act as the main determinant of rethrombosis. Because aggrastat has a short half life, sequential administration of aggrastat followed by rt-PA may be more effective than concomitant use of the two agents, which is supported by our results.

Statins are known to be effective for prevention of cardiovascular events.[18] Their beneficial effects are ascribed to lipid lowering effects and pleiotropic actions including the anti-thrombotic and anti-inflammatory actions.[19,20,23,33,34] Chronic administration of atorvastatin reduces plasma PAI-I concentration and PAI-I expression in adipose tissue.[35] In contrast to the lipid lowering effect which requires 2-4 weeks after administration of the statin to begin, the lipid-independent effects can be observed during an acute phase. Although there are few data regarding the safety or efficacy of statins administrated immediately after stroke onset, administration of rt-PA and statin shortly after cerebral infarction in an animal model reduced the infarction size and was associated with a positive outcome.[23] Some clinical studies have suggested that statin use with or without rt-PA in acute stroke patients can be safe and beneficial.[36,37]

Recent meta-analysis showed that prior statin use was neither an independent predictor of functional outcome nor of intracerebral hemorrhage in ischemic

stroke patients receiving IV rt-PA treatment. However, this particular meta-analysis focused on the neuroprotective effects of statin, not on the recanalization efficacy.[38] In our study, we investigated the effect of statin on recanalization, where it showed beneficial effects as an additional therapy in mice treated with rt-PA. Our findings suggest that use of statin in selected patients who are treated with rt-PA may improve clinical outcomes as successful recanalization is a good predictor of favorable outcome.[2]

Our data from ROTEM and platelet aggregometry imply that statins loosen the thrombus and enhance the thrombolytic activity of rt-PA, which is mediated by inhibition of the platelet-related pathway. At initial thrombus formation, low thrombin levels are required for making a porous fibrin scaffold, a process which is affected by extrinsic activity.[39] However, when the thrombus grows, the fibrin network continues to evolve with the addition of new fibers, and subsequently, the thrombus is larger and more integrated. Platelet-associated intrinsic procoagulant activities are involved during this phase.[39] Platelet inhibition by atorvastatin might prevent ongoing thrombi formation when rt-PA is administered.

Although ROTEM was developed for assessment of patient coagulation,

hemostatic therapy, transfusion requirements, blood loss during cardiac surgery, and massive trauma,[40] currently there are increasing interests in the application of ROTEM for evaluation of the hypercoagulable state in thromboembolic disorders. The hypercoagulable state as defined by thromboelastography can be used as a predictor of postoperative thromboembolic events.[41] While no information is available in the literature regarding use of ROTEM in ischemic stroke patients, our study suggests that methods using this device would give more information regarding the characteristics of thrombi and predictors of successful recanalization in ischemic stroke patients.

V. CONCLUSION

Aggrastat improved thrombolytic efficacy in mice treated with rt-PA. Our findings suggest that the administration timing of aggrastat has a significant effect. Aggrastat was effective when it was administrated sequentially following rt-PA infusion. Atorvastatin, a HMG-Co A reductase, also improved the efficacy of rt-PA. The beneficial effects of aggrastat and atorvastatin were associated with their antiplatelet actions, which resulted in prevention of re-occlusion after rt-PA treatment and suggesting that appropriate adjuvant treatment during IV rt-PA therapy may improve clinical outcome by potentiating successful recanalization.

VI. REFERENCES

1. Adams HP, Jr., del Zoppo G, Alberts MJ, Bhatt DL, Brass L, Furlan A, et al. Guidelines for the early management of adults with ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council, Clinical Cardiology Council, Cardiovascular Radiology and Intervention Council, and the Atherosclerotic Peripheral Vascular Disease and Quality of Care Outcomes in Research Interdisciplinary Working Groups: the American Academy of Neurology affirms the value of this guideline as an educational tool for neurologists. Stroke 2007;38:1655-711.
2. Rha JH, Saver JL. The impact of recanalization on ischemic stroke outcome: a meta-analysis. Stroke 2007;38:967-73.
3. Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. N Engl J Med 1995;333:1581-7.
4. Riedel CH, Zimmermann P, Jensen-Kondering U, Stingele R, Deuschl G, Jansen O. The importance of size: successful recanalization by intravenous thrombolysis in acute anterior stroke depends on thrombus length. Stroke 2011;42:1775-7.
5. Furlan A, Higashida R, Wechsler L, Gent M, Rowley H, Kase C, et al.

Intra-arterial prourokinase for acute ischemic stroke. The PROACT II study: a randomized controlled trial. Prolyse in Acute Cerebral Thromboembolism. JAMA 1999;282:2003-11.

6. Smith WS, Sung G, Saver J, Budzik R, Duckwiler G, Liebeskind DS, et al. Mechanical thrombectomy for acute ischemic stroke: final results of the Multi MERCI trial. Stroke 2008;39:1205-12.

7. Costalat V, Machi P, Lobotesis K, Maldonado I, Vendrell JF, Riquelme C, et al. Rescue, combined, and stand-alone thrombectomy in the management of large vessel occlusion stroke using the solitaire device: a prospective 50-patient single-center study: timing, safety, and efficacy. Stroke 2011;42:1929-35.

8. Medcalf RL. Desmoteplase: discovery, insights and opportunities for ischaemic stroke. Br J Pharmacol 2012;165:75-89.

9. Heo JH, Lee KY, Kim SH, Kim DI. Immediate reocclusion following a successful thrombolysis in acute stroke: a pilot study. Neurology 2003;60:1684-7.

10. Molina CA, Montaner J, Arenillas JF, Ribo M, Rubiera M, Alvarez-Sabin J. Differential pattern of tissue plasminogen activator-induced proximal middle cerebral artery recanalization among stroke subtypes. Stroke 2004;35:486-90.

11. Nam HS, Kim EY, Kim SH, Kim YD, Kim J, Lee HS, et al. Prediction of thrombus resolution after intravenous thrombolysis assessed by CT-based thrombus imaging. Thromb Haemost 2012;107.

12. Alexandrov AVMD, Grotta JCMD. Arterial reocclusion in stroke patients treated with intravenous tissue plasminogen activator. Neurology 2002;59:862-7.

13. Grotta JC, Welch KM, Fagan SC, Lu M, Frankel MR, Brott T, et al. Clinical deterioration following improvement in the NINDS rt-PA Stroke Trial. Stroke 2001;32:661-8.

14. Nordt TK, Moser M, Kohler B, Ruef J, Peter K, Kubler W, et al. Augmented platelet aggregation as predictor of reocclusion after thrombolysis in acute myocardial infarction. Thromb Haemost 1998;80:881-6.

15. Uyttenboogaart M, Koch MW, Koopman K, Vroomen PC, De Keyser J, Luijckx GJ. Safety of antiplatelet therapy prior to intravenous thrombolysis in acute ischemic stroke. Arch Neurol 2008;65:607-11.

16. Saposnik G, Di Legge S, Webster F, Hachinski V. Predictors of major neurologic improvement after thrombolysis in acute stroke. Neurology 2005;65:1169-74.

17. Mendonca N, Rodriguez-Luna D, Rubiera M, Boned-Riera S, Ribo M,

Pagola J, et al. Predictors of tissue-type plasminogen activator nonresponders according to location of vessel occlusion. Stroke 2012;43:417-21.

18. Amarenco P, Labreuche J. Lipid management in the prevention of stroke: review and updated meta-analysis of statins for stroke prevention. Lancet Neurol 2009;8:453-63.

19. Laufs U, Gertz K, Huang P, Nickenig G, Bohm M, Dirnagl U, et al. Atorvastatin upregulates type III nitric oxide synthase in thrombocytes, decreases platelet activation, and protects from cerebral ischemia in normocholesterolemic mice. Stroke 2000;31:2442-9.

20. Takemoto M, Liao JK. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors. Arterioscler Thromb Vasc Biol 2001;21:1712-9.

21. Zhang L, Zhang ZG, Ding GL, Jiang Q, Liu X, Meng H, et al. Multitargeted effects of statin-enhanced thrombolytic therapy for stroke with recombinant human tissue-type plasminogen activator in the rat. Circulation 2005;112:3486-94.

22. Ni Chroinin D, Callaly EL, Duggan J, Merwick A, Hannon N, Sheehan O, et al. Association between acute statin therapy, survival, and improved functional outcome after ischemic stroke: the North Dublin

Population Stroke Study. Stroke 2011;42:1021-9.

23. Zhang L, Chopp M, Jia L, Cui Y, Lu M, Zhang ZG. Atorvastatin extends the therapeutic window for tPA to 6 h after the onset of embolic stroke in rats. J Cereb Blood Flow Metab 2009;29:1816-24.

24. Korninger C, Collen D. Studies on the specific fibrinolytic effect of human extrinsic (tissue-type) plasminogen activator in human blood and in various animal species in vitro. Thromb Haemost 1981;46:561-5.

25. Delsing DJ, Jukema JW, van de Wiel MA, Emeis JJ, van der Laarse A, Havekes LM, et al. Differential effects of amlodipine and atorvastatin treatment and their combination on atherosclerosis in ApoE*3-Leiden transgenic mice. J Cardiovasc Pharmacol 2003;42:63-70.

26. Schochl H, Posch A, Hanke A, Voelckel W, Solomon C. High-dose fibrinogen concentrate for haemostatic therapy of a major trauma patient with recent clopidogrel and aspirin intake. Scand J Clin Lab Invest 2010;70:453-7.

27. Adams HP, Jr., Effron MB, Torner J, Davalos A, Frayne J, Teal P, et al. Emergency administration of abciximab for treatment of patients with acute ischemic stroke: results of an international phase III trial: Abciximab in Emergency Treatment of Stroke Trial (AbESTT-II). Stroke 2008;39:87-99.

28. Siebler M, Hennerici MG, Schneider D, von Reutern GM, Seitz RJ, Rother J, et al. Safety of Tirofiban in acute Ischemic Stroke: the SaTIS trial. Stroke 2011;42:2388-92.

29. Fassbender K, Dempfle CE, Mielke O, Schwartz A, Daffertshofer M, Eschenfelder C, et al. Changes in coagulation and fibrinolysis markers in acute ischemic stroke treated with recombinant tissue plasminogen activator. Stroke 1999;30:2101-4.

30. Kawano K, Aoki I, Aoki N, Homori M, Maki A, Hioki Y, et al. Human platelet activation by thrombolytic agents: effects of tissue-type plasminogen activator and urokinase on platelet surface P-selectin expression. Am Heart J 1998;135:268-71.

31. Kwon JH, Shin SH, Weon YC, Hwang JC, Baik SK. Intra-arterial adjuvant tirofiban after unsuccessful intra-arterial thrombolysis of acute ischemic stroke: preliminary experience in 16 patients. Neuroradiology 2011;53:779-85.

32. Christou I, Alexandrov AV, Burgin WS, Wojner AW, Felberg RA, Malkoff M, et al. Timing of recanalization after tissue plasminogen activator therapy determined by transcranial doppler correlates with clinical recovery from ischemic stroke. Stroke 2000;31:1812-6.

33. Reiss AB, Wirkowski E. Role of HMG-CoA reductase inhibitors in

neurological disorders : progress to date. Drugs 2007;67:2111-20.

34. Mihos CG, Salas MJ, Santana O. The pleiotropic effects of the hydroxy-methyl-glutaryl-CoA reductase inhibitors in cardiovascular disease: a comprehensive review. Cardiol Rev 2010;18:298-304.

35. Li JQ, Zhao SP, Li QZ, Cai YC, Wu LR, Fang Y, et al. Atorvastatin reduces plasminogen activator inhibitor-1 expression in adipose tissue of atherosclerotic rabbits. Clin Chim Acta 2006;370:57-62.

36. Cappellari M, Deluca C, Tinazzi M, Tomelleri G, Carletti M, Fiaschi A, et al. Does statin in the acute phase of ischemic stroke improve outcome after intravenous thrombolysis? A retrospective study. J Neurol Sci 2011;308:128-34.

37. Flint AC, Kamel H, Navi BB, Rao VA, Faigeles BS, Conell C, et al. Statin use during ischemic stroke hospitalization is strongly associated with improved poststroke survival. Stroke 2012;43:147-54.

38. Engelter ST, Soinne L, Ringleb P, Sarikaya H, Bordet R, Berrouschot J, et al. IV thrombolysis and statins. Neurology 2011;77:888-95.

39. Wolberg AS. Thrombin generation and fibrin clot structure. Blood Rev 2007;21:131-42.

40. Bolliger D, Seeberger MD, Tanaka KA. Principles and practice of thromboelastography in clinical coagulation management and

transfusion practice. Transfus Med Rev 2012;26:1-13.

41. Dai Y, Lee A, Critchley LA, White PF. Does thromboelastography predict postoperative thromboembolic events? A systematic review of the literature. Anesth Analg 2009;108:734-42.

ABSTRACT (In Korean)

혈전용해치료 모델을 통한 적절한 혈전용해 치료 효능 비교

<지도교수 허 지 회>

연세대학교 대학원 의학과

김 영 대

정맥내 인간재조합 조직 플라스미노겐 활성화인자(rt-PA) 투여는 급성 뇌경색에서 효과적인 치료이지만, 아직 그 효과가 기대에 미치지 못하고 있다. 혈전용해치료 중 혈전은 용해와 재형성을 반복하는 역동적인 과정을 겪으며, 혈전재형성 과정에는 혈소판이 중요한 역할을 한다. 본 연구에서는 아그라스타트와 아토바스타틴을 rt-PA와 병용투여시 혈관재관통의 효능을 증진시키는지 검증하였다. 생체내 모

델로, 염화제이철($FeCl_3$)에 의해 유도되는 마우스 동맥 내 혈전생성 모델을 통해 초음파 혈류량계를 이용하여 실시간으로 혈류량을 측정함으로써 혈관재관통 여부를 평가했고, 생체외 모델로, rotation thromboelastometry (ROTEM) 과 혈소판 응집측정계을 이용하여 혈전의 특성 및 혈소판의 기능 억제여부를 확인하였다. 아그라스타트를 rt-PA 투여 직후에 병용투여시에는 혈전의 재협착을 억제하여 성공적인 혈전재관통률을 증가시켰다. 하지만 아그라스타트와 rt-PA의 동시 투여는 혈전용해효능을 증강시키지 못함이 관찰되었다. rt-PA 단독 투여에 비해서 아토바스타틴과 rt-PA를 동시 투여한 경우에는 혈전용해 시작 및 재관통까지 걸리는 시간을 단축시켰으며 혈전재형성까지의 시간을 연장시켰다. ROTEM 을 이용한 연구에서 아토바스타틴과 rt-PA의 병용투여군에서 EXTEM 분석상 20, 25, 30분째 혈전의 굳기(firmness)를 약하게 함이 관찰되었고, 혈전용해가 더 많이 이루어지는 것이 관찰되었지만 INTEM 분석상에서는 병용투여군과 rt-PA 단독 투여군간의 차이는 관찰되지 않았다. 이는 아토바스타틴과 rt-PA의 병용투여로 인한 혈전용해능의 향상은 혈소판의 작용을 억제함으로써

이루어짐을 시사한다. 혈소판 응집측정계를 이용한 분석에서는 아토바스타틴의 급성 투여시 최대 응집값(maximal aggregation value)이나 area under curve에서 의미있게 작았고 이는 아토바스타틴의 투여 초기에 혈소판의 기능을 억제함을 확인한 것이다. 하지만 혈중 지질농도 분석상에서는 아토바스타틴의 급성투여군과 대조군간에 의미있는 차이를 보이지 않았다. 본 연구는, rt-PA와 적절한 약제의 병용투여를 통해서 혈전용해제를 통한 성공적인 혈관재관통의 효과를 상승시킬 수 있으며, 결국 환자의 예후를 향상시킬 수 있음을 시사한다.

핵심되는 말: 정맥내 인간재조합 조직 플라스미노겐 활성화인자, 혈전용해치료, 항당단백IIb/IIIa 제재, 지질저하제

PUBLICATION LIST

1. Kim YD, Lee JH, Jung YH, Cha MJ, Choi HY, Nam CM, Yang JH, Chol HJ, Nam HS, Heo JH. Effect of warfarin withdrawal on thrombolytic treatment in patients with ischaemic stroke. Eur J Neurol 2011;18(9):1165-70.

2. Kim YD, Lee JH, Jung YH, Cha MJ, Choi HY, Nam CM, Yang JH, Cho HJ, Nam HS, Lee KY, Heo JH. Increases in cerebral atherosclerosis according to CHADS2 scores in patients with stroke with nonvalvular atrial fibrillation. Stroke. 2011;42(4):930-4.

3. Kim YD, Cha MJ, Kim J, Lee DH, Lee HS, Nam CM, Nam HS, Heo JH. Ischaemic cardiovascular mortality in patients with non-valvular atrial fibrillation according to $CHADS_2$ score. Thromb Haemost. 2011;105(4):712-20.

4. Kim YD, Lee JH, Jung YH, Choi HY, Nam CM, Yang JH, Cho HJ, Nam HS, Lee KY, Heo JH. Safety and outcome after thrombolytic treatment in ischemic stroke patients with high-risk cardioembolic sources and prior subtherapeutic warfarin use. J Neurol Sci. 2010 ;298(1-2):101-5.

5. Kim YD, Park B, Cha MJ, Nam CM, Nam HS, Ha JW, Chung N, Heo JH. Stroke severity in concomitant cardiac sources of embolism in patients with atrial fibrillation. J Neurol Sci. 2010;298(1-2):23-7.

6. Kim YD, Choi HY, Cho HJ, Cha MJ, Nam CM, Han SW, Nam HS, Heo JH.

Increasing frequency and burden of cerebral artery atherosclerosis in Korean stroke patients. Yonsei Med J. 2010;51(3):318-25.

7. Kim YD, Choi HY, Jung YH, Nam CM, Yang JH, Cho HJ, Nam HS, Lee KY, Heo JH. Mirror pattern of cerebral artery atherosclerosis in patients with ischaemic stroke. Eur J Neurol. 2009 ;16(10):1159-64.

8. Kim YD, Choi HY, Jung YH, Nam CM, Yang JH, Cho HJ, Nam HS, Lee KY, Heo JH. Classic risk factors for atherosclerosis are not major determinants for location of extracranial or intracranial cerebral atherosclerosis. Neuroepidemiology. 2009;32(3):201-7.

9. Hong HJ, Kim YD, Cha MJ, Kim J, Lee DH, Lee HS, Nam CM, Nam HS, Heo JH. Early neurological outcomes according to $CHADS_2$ score in stroke patients with nonvalvular atrial fibrillation. Eur J Neurol. 2012 ;19(2):284-90.

10. Kim YD, Hong HJ, Cha MJ, Nam CM, Nam HS, Heo JH. Determinants of infarction patterns in cardioembolic stroke. Eur Neurol 2011;66(3):145-50.