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Efficacy of Lumbrokinase and Warfarin Compared to Single Warfarin on Thrombus Resolution in Deep Vein Thrombosis

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Abstract

Background: Deep vein thrombosis (DVT) is a challenging condition for clinician in all specialities. Prognosis after vein thromboembolism is worse and much worse after pulmonary embolism. Anticoagulant is the mainstay therapy for deep vein thrombosis, but there is still slow thrombus resolution even with the use of optimal anticoagulant. The use of intravenous thrombolytic agents is one of the methods to significantly lyse thrombus. Since there is increasing risk of bleeding with the use of the agents, indication is limited. Lumbrokinase is oral thrombolytic that may give significant thrombus lyses without increasing the risk of bleeding for deep vein thrombosis. This study was conducted to compare single warfarin therapy with combination of lumbrokinase and warfarin for thrombus resolution in deep vein thrombosis patients.

Methods: This study was a randomized open labeled trial comparing deep vein thrombosis patients using single warfarin therapy group to group using combination lumbrokinase and warfarin. 22 patients meet the inclusion and exclusion criteria. Patients were followed for 30 days and in the end of the trial, evaluation using vascular Doppler ultrasonography was done. Chi-square analysis was used to compare the outcome between two therapy groups.

Results: In this trial, group therapy with added lumbrokinase to warfarin yielded a tendency toward better thrombus resolution compared to group with single warfarin therapy (58.3% vs. 30%, p=0.231).

Conclusion : Added therapy with lumbrokinase to warfarin may give better thrombus resolution as compared to single warfarin therapy, although there is no significant difference between groups.

Keywords: deep vein thrombosis, lumbrokinase, warfarin

Introduction

Deep vein thrombosis (DVT) is a challenging condition for all clinicians. It could happen in every vein system area with main attention to the development of fatally complication known as pulmonary embolism (PE).[1] The mainstay therapy of DVT is to cease the development of thrombus and prevent recurrence and thromboemboli complication. All of these goals are achieved using anticoagulants.[2] The use of thrombolytic agent in DVT has not yet being examined. Thrombolytic agents could produce faster thrombus lyses and recanalyzation of occluded veins, as well as to reduce symptoms. Although the improved outcome, there were only few studies that have been conducted to show better clinical improvement by using thrombolytic agents compared to anticoagulants.[3]

Lumbrokinase is oral thrombolytic agents introduced by Mihara in 1991. Lumbrokinase came from Lumbricidae families of earthworm that has properties to dissolute fibrin and activate plasminogen. Active substance from these earthworms that had been characterized and known to have thrombolytic properties was serine protease enzyme group. This enzyme was known to have thrombolytic and fibrinogenolytic properties, lower blood viscosity and degrade thrombus. [4] With all of these properties, lumbrokinase could be a promising agent in patients with DVT.

Lumbrokinase addition to warfarin therapy may increase thrombus resolution in DVT patients. Warfarin takes 5 days to achieve optimal anticoagulation, in the other hand, lumbrokinase can achieve fibrinolytic within hours and could be given orally. The use of anticoagulant only accomplishes 6% thrombus lyses in acute illiofemoralis and femoralis DVT within 10 days therapy. Thrombus propagation can also be found in 40% of patients that had already used heparin. [5]

The aim of this study is to compare the efficacy of lumbrokinase and warfarin and single warfarin in DVT patients. A primary outcome

is thrombus resolution in both treatments and a secondary outcome is safety parameters in lumbrokinase and warfarin therapy.

Method

This open-labeled randomized control trial has been conducted in RSUP Dr. Sardjito Yogyakarta from Mei to December 2014 with DVT patients as study subjects. Inclusion criteria are: (1) DVT patients diagnosed by Doppler vascular ultrasonography, (2) patients age between 30 to 70 years and (3) patients agree to participate in this study by signing informed consents. Exclusion criteria are: (1) patients with increased level of aminotransferase enzymes above twice the upper limit, (2) patients with chronic kidney disease with creatinine clearance time <30 mL/minute (Cockroft-Gault criteria), (3) antiplatelet use, (4) bleeding abnormalities and active bleeding, (5) pregnancy and lactating, (6) uncontrolled hypertension (systolic blood pressure>180 mmHg and diastolic blood pressure>110 mmHg), and (7) critically ill patients or patients with severe infection.

Dependent variable in this study is resolution of thrombus. Independent variable is the use of lumbrokinase and warfarin and single warfarin therapy. Confounding variabless are the onset of symptoms, the coagulation functions and the underlying clinical condition, such as cancer or heart failure.

Study Protocol

Patients with DVT who met inclusion criteria would sign an informed consent to participate in this study and be admitted to DVT treatment in study protocol. Subjects were randomized after five days of i.v UFH and initial warfarin therapy. Subjects were then divided into two groups, either lumbrokinase and warfarin therapy group or single warfarin group.

Anamnesis on history of the disease and risk factor, physical examination, thorax photo, laboratory examinations (complete blood count, renal function test, liver function test, fasting

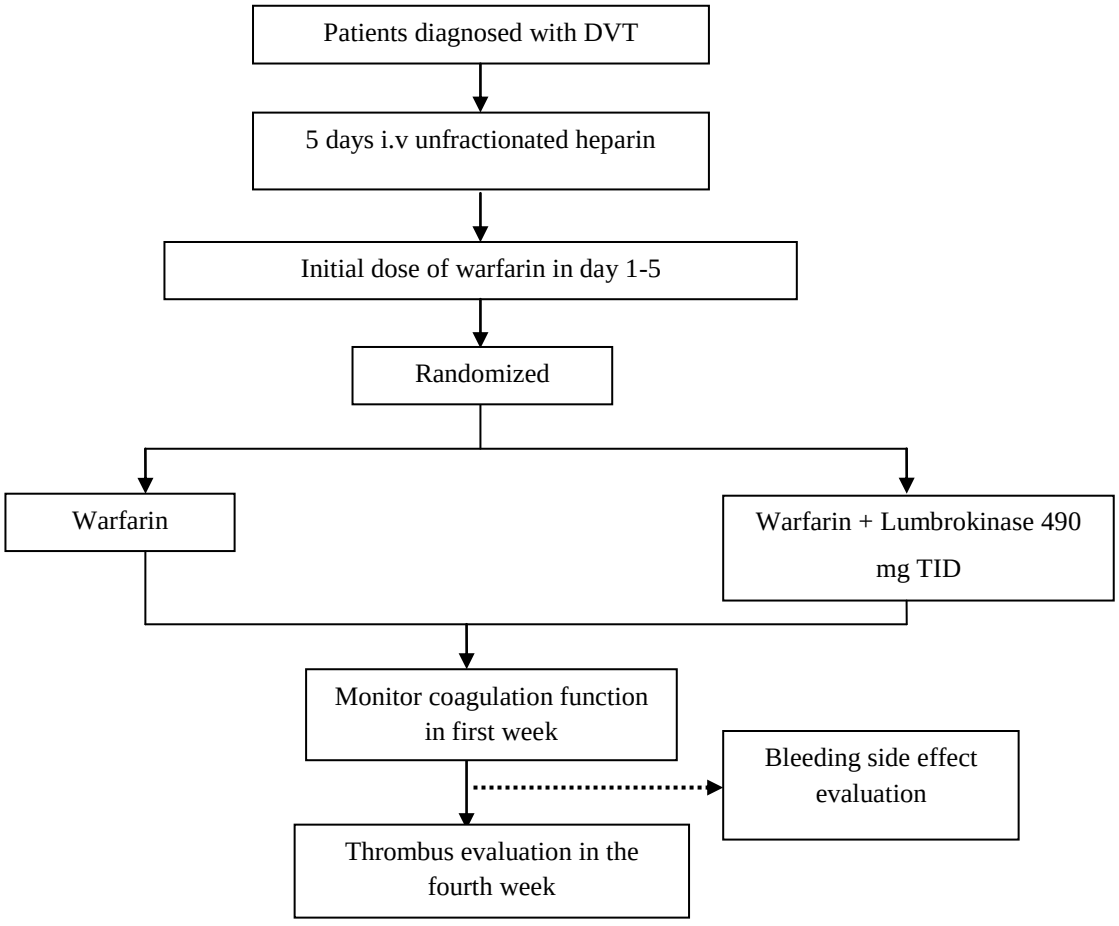

Figure 1.Study protocol

blood glucose, HbA1c in patient with history of diabetes mellitus, complete cholesterol workup, and coagulation function) were done in all of the subjects. Subjects were randomized in the end of the fifth day to determine which therapy they would be admitted, using combination of lumbrokinase and warfarin or single warfarin therapy until the end of the fourth week.

Coagulation functions were monitored in the first five days of warfarin initiation and intravenous (i.v) unfractionated heparin therapy. The monitoring in the first five days consisted of routine 3, 6, 12 and 24 hour aPTT check and in interval of 24 hours. International normalized ratio (INR) was evaluated in day three for dose adjustment to achieve target INR 2-3. Afterward, coagulation function were measured in the end of the fourth week.

Doppler vascular ultrasonography was conducted initially to determine the diagnosis of DVT and in the end of the study to evaluate the effect of the therapy. The locations of the thrombus, diameter thrombus, compression ultrasonography and flow pattern were items that were taken in the test.

Resolution of the thrombus was measured using lytic thrombus criteria by Protack *et al* [6] Outcome of the study was divided into two categories, without improvement and resolution. Without improvement was define as propagation of thrombus to other vein segment or if thrombus was seen in the same segment with <50% change in diameter of thrombus. Resolution was define if there was decreasing diameter of thrombus ≥ 50% or if thrombus could not be visualized in the same vein segment.

Subjects were asked to report any adverse reactions such as allergy or any minor or major bleeding. Any major bleeding or severe adverse reaction would halt lumbrokinase or warfarin therapy.

Statistical analysis

Continuous data between groups were compared by unpaired T test. Categorical data between groups were compared with chi-square test. In the 2x2 tables, Fisher exact test was used when Chi-Square test requirements were not staisfied. Multivariate analyses were performed to examine whether cancer and heart failure influenced the resolution of thrombus. Analysis considered significant when the p value <0.05. Data analysis was performed with SPSS version 22. Approval from the Medical and Health Research Ethics Committee, Faculty of Medicine, University of Gadjah Mada, was given before the research was conducted.

Table1. Baseline characteristic of the subjects between groups

Variables	Warfarin and Lumbrokinase (n=12)	Warfarin (n=10)	*P* value
Age (mean,years)	50.67±12.62	53.9±13.3	0.567
Gender, n(%)			
Male	8 (36%)	2 (9%)	0.029
Female	4 (18%)	8 (36%)	
Onset of symptom, n(%)			
Acute (< 2 weeks)	7(46.7%)	3(42.9%)	1.00
Chronic (>2 weeks)	8(53.3%)	4(57.1%)	
BUN, mean±SB, (mg/dL)	13.7±8.52	10.27±4.21	0.252
Creatinine, mean ±SB, (mg/dL)	1.19±0.79	0.83±0.36	0.210
SGOT, mean±SB (U/L)	32.6 ± 13.1	24.1±8.9	0.097
SGPT, mean±SB (U/L)	32.0 ± 12.7	18.0 ± 9.0	0.009
Coagulation function, mean±SB			
aPTT	35.7 ± 5.8	44.6 ± 29.7	0.317
PPT	15.4 ± 2.2	16.0 ± 1.8	0.500
INR	1.10 ± 0.20	1.19 ± 0.15	0.396
Underlying condition, n(%)			
Imobilization	2(17%)	5(50%)	0.095
Malignancies	1(8%)	5(50%)	0.029
Heart failure	0	3(30%)	0.041
Post operative	1(8%)	0	0.350
Hormonal contraceptive	1(8%)	0	0.350

Results

Baseline characteristic of the subjects

This study was conducted in Mei-December 2014 in Cardiovascular Care Unit RSUP Dr. Sardjito, Yogyakarta. A total of 25 subjects met inclusion criteria but there were drop out in 3 subjects due to warfarin allergy (n=1) and loss of follow up (n=2). The baseline characteristic of subjects were presented in table 1.

Deep vein thrombus resolution

Doppler vascular ultrasonography were performed in 22 subjects during initial examination and post therapy. The results of the examination showed thrombus in femoral veins (12 subjects (54.5%)), popliteal veins (9 subjects (40.9%) and subclavian vein (1 subject (4.5%)). There were no significant difference between two treatment groups in regard of the location of the thrombus (p=0.529). Figure 2 showed the percentage of thrombus location.

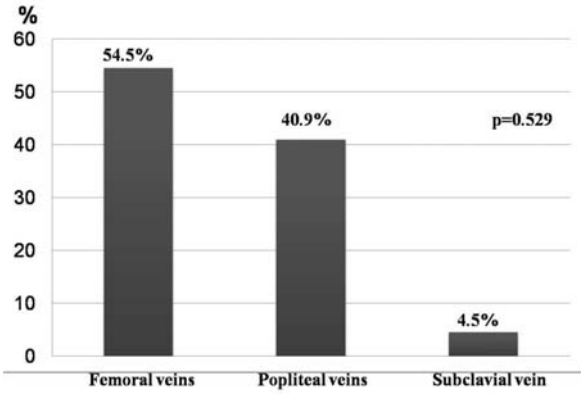

Figure 2. Distribution of thrombus location among subjects

Mean diameter of the thrombus in initial diagnosis was 3 mm (range: 1-12 mm) with mean diameter in lumbrokinase and warfarin group was 4.75 ± 3.84 mm and mean diameter in single warfarin group was 3.6 ± 1.89 mm. There was no significant difference in initial thrombus diameter between two therapy groups (p=0.399). Mean diameter of thrombus during evaluation was 2.5 mm (range: 0-12 mm) with thrombus resolution was 44.5% (range: 0-100%).

Figure 3 showed the percentage of subjects with thrombus resolution compared to those without improvement. In both treatment groups there were 45.5% subjects with resolution and 54.5% subjects without improvement.

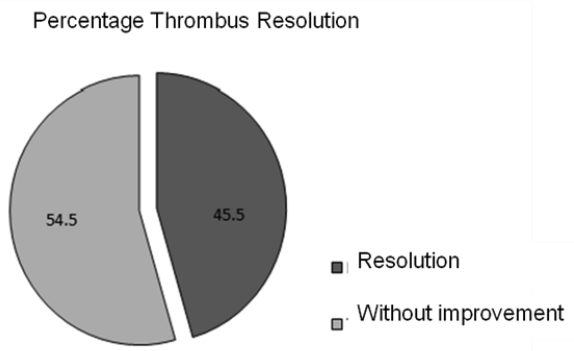

Figure 3. Percentage of subjects with resolution and without improvement in both treatment groups

Mean thrombus resolution in lumbrokinase and warfarin group was 53.2% ± 39.0% and in single warfarin group was 30.0% ± 40.0% (p=0.203). Mean percentage of thrombus resolution in lumbrokinase and warfarin group was better as compared to its resolution in single warfarin group, although there was no significant difference. Figure 4 showed the mean thrombus resolution between two therapy group.

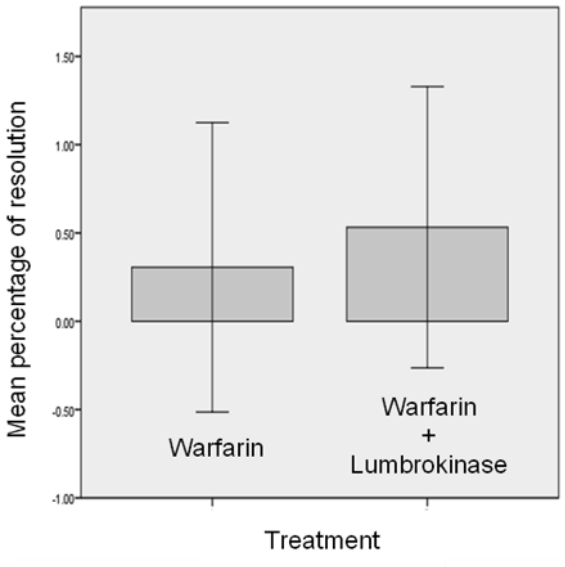

Figure 4. Mean percentage of thrombus resolution in single warfarin group compared to lumbrokinase and warfarin group

The comparison between the number of subjects with resolution in lumbrokinase and warfarin group and single warfarin group was analyzed using Chi-square. Table 2 showed the comparison of resolution between two therapy groups.

Table 2. Comparison between between subjects with resolution in lumbrokinase and warfarin group to single warfarin group

Categories	Warfarin + Lumbrokinase	Warfarin	p value	OR	95% CI
Resolution (%)	58.3	30.0	0.231	3.26	0.55-19.25
Without improvement (%)	41.7	70.0			

Table 3. Renal function test, liver function test and coagulation function in two treatment groups before and after therapy

Variable	Before therapy	After therapy	p value
Warfarin			
BUN (mg/dL)	10.3±4.2	19.6±7.1	0.012
Creatinin (mg/dL)	0.8±0.4	1.0±0.5	0.032
SGOT (U/L)	24.1±8.9	23.4±12.2	0.674
SGPT (U/L)	18.0±9.0	16.4±8.3	0.401
APTT (detik)	44.7±29.7	38.9±18.4	0.646
PPT (detik)	16.3±1.8	21.9±17.6	0.440
INR	1.2±0.1	1.8±1.7	0.114
Warfarin and lumbrokinase			
BUN (mg/dL)	13.7±8.5	12.6±8.5	0.170
Creatinin (mg/dL)	1.19±0.79	0.9±0.2	0.722
SGOT (U/L)	32.6±13.1	29.4±15.2	0.182
SGPT (U/L)	32.0±12.7	26.4±17.0	0.054
APTT (detik)	35.7±5.8	40.1±7.1	0.050
PPT (detik)	15.4 ± 2.1	23.1 ± 6.5	0.020
INR	1.12 ± 0.2	2.0 ± 0.8	0.010

Table 2. showed that percentage number of subjects with resolution were tend to be larger in lumbrokinase and warfarin group (70.0% vs 30.0%), although there was no significance difference (p=0,231). Odds ratio in resolution of the thrombus using lumbrokinase and warfarin was 3.26 (95% CI 0.55-19.25), however no significant.

Safety parameter

In this study, we conducted assessments of bleeding effect in both treatment groups. During the study, there was no report on adverse event in all of study subjects.

All subjects received warfarin with therapeutic target of INR 2-3. An INR target was not achieved in as many as 72.7% subjects although the evaluation of INR was done routinely and warfarin dosing was adjusted. There were 66.7% of subjects (n=8) with INR below the target, in whom the evaluation showed without improvement of thrombus. Figure 5 showed comparison of INR in patients without thrombus improvement.

The use of lumbrokinase in clinical trial was limited and there was no study that measure

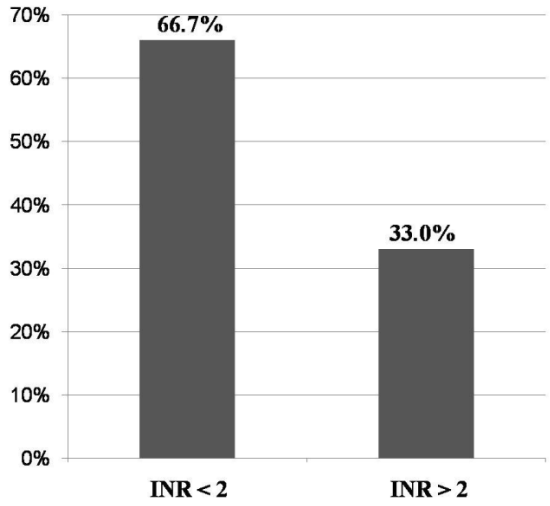

Figure 5. Comparison of INR value in without improvement group

laboratory parameter in patients with DVT. In this study we measured renal function test, liver function test and coagulation function post lumbrokinase and warfarin therapy and single warfarin therapy. Table 3 showed comparison in two treatment groups before and after therapy.

Discussion

In this study there were 10 male subjects (40%) and 12 female subjects (48%) with mean age of 52.14 years (SD± 12.7 years). In patients > 40 years old there were increased risk of having DVT which is consistent with the study findings.[7] Thrombus distribution in this study was mainly in proximal vein, mostly in femoral vein. In distal thrombus, symptoms were rarely meet. Proximal vein involvement was as many as 89% in the previous study of 189 patients with symptomatic DVT.[8]

Mean thrombus resolution in this study was 44.5%, which was similar with finding in the studies by Killewich et al. and Caprini et al. that stated there were 45% until 56% of thrombus resolution within 30 days post anticoagulation therapy.[9,10] In this study, the percentage of subjects with resolution were larger in lumbrokinase and warfarin therapy group compared to single warfarin group although there were no sinificant difference. This could be attributd to the lyses effect of thrombus by lumbrokinase that was added to standard warfarin therapy. The use of anticoagulant alone did not give thrombus lyses property nor breakdown of the clot. The breakdown of the clot depends on the thrombolytic ability in every individual. Thus, lumbrokinase which works by degrading thrombus directly could help in speed thrombus lyses process and enhanced thrombus resolution.

Jin *et al.* showed increased level of tissue plasminogen activator (tPA) in patients with cerebral infarction that were given lumbrokinase. Hence, in this study increasing tPA level could be the mechanism that enhanced thrombolytic effect in patients with DVT. [11] Recanalization of thrombus in DVT patient was seen in case report by Kaligis who determined clinical improvement and full recanalization in right common femoralis veins after lumbrokinase use in 14 days. [12] This study showed the potentially better thrombus resolution in adding lumbrokinase to standard warfarin therapy as the mainstay therapy for DVT.

There were no significant difference in subjects with resolution between two therapy groups in this study. This could be cause by the small number of subjects obtained in this study and the short observation time. Also in our study there were subjects with malignancy and heart failure. Heart failure had 0.67 odds ratio to achieve thrombus resolution (95% CI: 0.01-9.19). Malignancies that occured in 5 subjects (23%) were all failed to show thrombus resolution. Hence malignancies were one of the condition that cause failure to achieve thrombus resolution.

In malignancies patient, residual thrombus that were shown with CUS abnormalities were higher compared to non malignancies. As many as 23% of patients with malignancies had CUS normalization in 6 months compared to 53% patients in non malignancies patients.[13] This condition could be due predisposing factor that stayed even after therapy which is prothrombotic condition in malgnancies and the difficulty of management of anticoagulant due to difficulty of monitoring. This condition was caused by changes of nutritional status, multiple drugs therapy and change of metabolism.[13,14] Heart failure was also a predictor of vein thromboembolism. But,studies on residual thrombus in heart failure patients were only few. Piazza *et al*. studied 1822 heart failure patients with thromboemboli and showed recurrency DVT in 37 patients due to interaction of multiple factors such as immobilization, acute infection, chronic lung disease, stroke and other condition.[15]

In safety parameter there were no bleeding side effect observed during the study. Assessment of hepatic and renal function were also showed that adding lumbrokinase to warfarin therapy did not alter the hepatic and renal function. This findings were consistent with finding by Gayatri*et al*. in healthy subjects. [16]

The difference between this study and other study that showed no difference in aPTT, PPT and INR value could be to the effect of the use of warfarin. By observing therapy effect of warfarin to aPTT,PPT and INR also the increasing aPTT value in lumbrokinase therapy, we conclude that increased coagulation function observed post therapy of warfarin and lumbrokinase could be due to interaction betwee two therapies.

Study Limitation

In this study, the sample size was smaller than expected, also there were difficulty in achieving target INR in both groups. Other limitation of this study was the short observation time that cause inability to measure full resolution of thrombus.

Conclusion

In this study the percentage of subjects that had thrombus resolution were larger in

lumbrokinase and warfarin group as compared to thrombus resolution in single warfarin group, although there were no significant difference.

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