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Improvement in Functional Status of Acute Ischemic Stroke Patients Treated with DLBS1033 as Add on Therapy : A Randomized Controlled Study

R. T. Pinzon[1], V. Veronica[2]*

[1] *Lecturer, department of neurology, faculty of medicine, Duta Wacana Christian University, Wahidin Sudirohusodo st. 5-25, Yogyakarta 55224, Indonesia*

[2] *Research assistant, department of neurology, faculty of medicine, Duta Wacana Christian University, Wahidin Sudirohusodo st. 5-25, Yogyakarta 55224, Indonesia*

Abstract

Background: DLBS0133 – a lumbrokinase fractionated from the earthworms, *Lumbricus rubellus* – was a promising agent in patients with ischemic stroke.

Aims: Measure the benefit of DLBS1033 as add on therapy for ischemic stroke patients.

Methods: This was randomized, controlled, open-label study from the period of November 2019 - April 2020 at Bethesda Hospital, Yogyakarta, Indonesia. The subjects were randomized into 2 groups: control group who received standard therapy or experimental group who received standard therapy and DLBS1033 3 times daily. Disabilities were measured with modified Rankin Scale (mRS), National Institutes of Health Stroke Scale (NIHSS) score, and Barthel Index (BI) between 2 groups. The analysis is intention to treat based.

Results: The data were obtained from 60 ischemic stroke patients, consist of 36 male and 24 female. The mean age was 61.9±12.462 years old. There were significant improvements in mRS (3.30±0.724 vs 1.78±0.974) (p <0.001), NIHSS (7.11±2.806 vs 2±2.787) (p <0.001) and BI (4.63±3.53 vs 14.52±5.199) (p <0.001) scores after treatment in experimental group. The improvements of mRS (3.11±0.801 vs 2.26±1.059) (p <0.001), NIHSS (6.19±2.788 vs 2.52±3.227) (p <0.001), and BI (6.59±4.693 vs 13.44± 5.905) (p <0.001) in control group were also significant. Differences of mRS (1.52±0.643 vs 0.85±0.534) (p <0,001), NIHSS (5.11±1.826 vs 3.67±1.941) (p: 0,012), and BI (9.96±3.716 vs 6.85±3.146) (p: 0,001) scores between 2 groups were significantly different.

Conclusion: DLBS1033 has proven to improve functional outcomes better than standard therapy. This study did not find any adverse events related to DLBS1033.

Keywords: DLBS1033, Standard therapy, lumbrokinase, Acute ischemic stroke, Functional outcomes.

INTRODUCTION

The second spot as the leading cause of death and the third spot as the leading cause of disability-adjusted life years (DALYs) lost in 2017 were claimed by stroke [1]. Stroke is more common in developing countries than developed countries [2]. Stroke's mortality and DALYs lost in Indonesia consecutively are 119.5/100,000 and 2,857.91/100,000 - the highest among any other country in South-East Asia [1].

Stroke can be divided into 2 main types, which are ischemic stroke and hemorrhagic stroke. Ischemic stroke occurs when blood supplies to brain are obstructed, meanwhile hemorrhagic stroke occurs when blood vessel bursts [3].

DLBS1033 contains lumbrokinase derived from the Lumbricus rubellus [4]. Japanese researchers were the first one to found fibrinolytic and fibrinogenolytic activities of lumbrokinase [5].

Excessive bleeding does not occur with DLBS1033 because of its specific fibrin properties [6]. Lumbrokinase has both antithrombotic and thrombolytic activities; thus, unlike the anticoagulants, lumbrokinase suppresses new clots formation and breaks up clots that have already been formed [4, 7]. Furthermore, DLBS1033 has been proven through safety studies in human [8, 9].

The aim of this study is measuring the benefit of DLBS1033 as add on therapy for ischemic stroke patients.

MATERIAL AND METHODS

Study Design

This was randomized, controlled, open-label, study from the period of November 2019 - April 2020 at Bethesda Hospital, Yogyakarta, Indonesia. Yogyakarta has the second highest stroke cases among provinces in Indonesia [10].

There were 60 acute ischemic stroke patients who fulfilled the inclusion and exclusion criteria. Each subject recruited from acute stroke intensive care unit had been followed up from the first day they were hospitalized until hospital discharge (died or discharged alive). Eligible subjects were randomly allocated to receive any of the following regiments: standard therapy consists of aspirin 100 mg once daily, atorvastatin 20 mg once daily, vitamin B12 100 mg three times daily (control group) or standard therapy and DLBS1033 3 times daily (experimental group).

Subject Selection

Sixty subjects consecutively recruited. The inclusion criteria of this study were: (i) Male or female, (ii) Adult age (>18 years old), (iii) Diagnosed with acute ischemic stroke for the first time, (iv) The onset is <24 hours, (v) Not a referral patient, (vi) GCS score of 15 (fully alert), and (viii) Mild to moderate scores on NIHSS. The exclusion criteria were: (i) Subjects known to have hypersensitivity to DLBS1033 (ii) Participated in other

studies for the past 1 month, and (iii) Not competent enough in giving approval and answering questionnaires.
Subjects were withdrawn from this study if: (i) subjects experienced any serious adverse events, (ii) subjects were suffering from any disease that would interfere with medication and evaluation, (iii) subjects died.

Ethical approval number 07/KEPK-RSB/I/20 was obtained from Bethesda Hospital, Yogyakarta, Indonesia. This research has been registered at Center for Health Resources and Services Research and Development Indonesia with the ethical approval number of 1087/C.16/FK/2019.

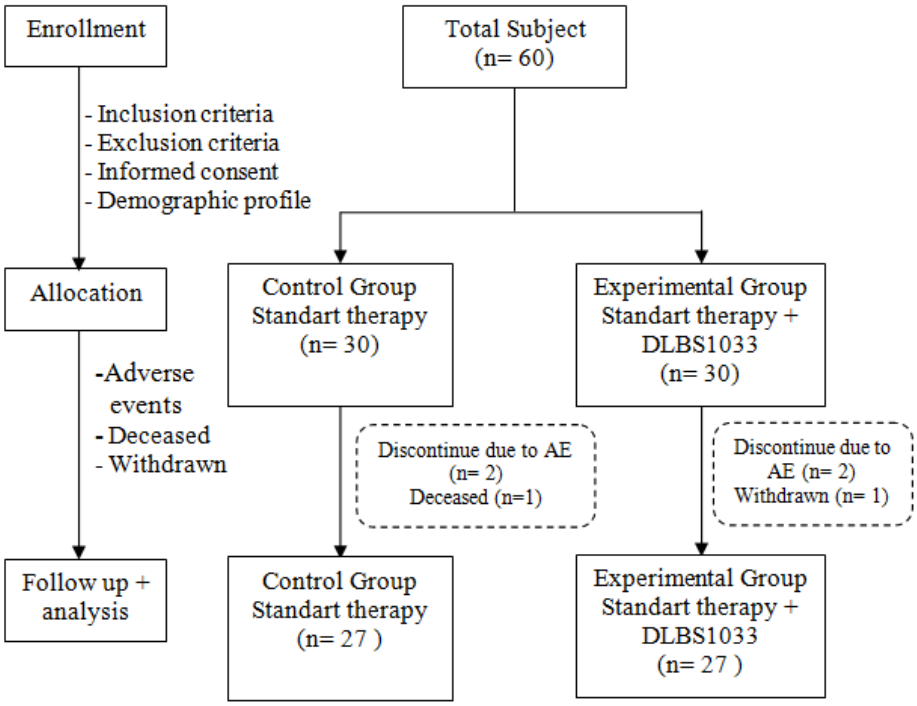

Figure 1: The flow diagram of the research

Outcomes Measurement

Disabilities on onset and hospital discharge were evaluated with modified Rankin Scale (mRS), National Institutes of Health Stroke Scale (NIHSS), and Barthel Index (BI). The mRS consists of 7-grade scale, including functional independence and gait with a score range of 0-6. Score of 6 represent the worst outcome. The NIHSS consists of 11 parameters with a score range of 0-42. The worst possible neurologic deficit is given a score of 42. The BI consists of 10 items, score of 0 represent a totally dependent patient, and meanwhile a score of 20 represent an independent patient [11].
Laboratory results evaluated in this study were total leukocyte count (TLC), hemoglobin (Hb), hematocrit (HCT), platelet count, total cholesterol, cholesterol LDL, urea, creatinine, sodium, potassium, and random blood glucose level. Adverse events were observed and recorded during study conduct.

Statistical Analysis

The analysis is intention to treat based. Subjects' characteristics were presented on descriptive analysis, chi-square, Mann-Whitney test, and independent t-test. Bivariate analysis using paired t-test and Wilcoxon test was conducted by analyzing improvement of mRS, NIHSS, and BI score in both experimental and control group. Mann-Whitney test was used for analyzing comparison of differences in mRS, NIHSS, and BI score between 2 groups. Chi-square test to compares the mRS and BI scores at hospital discharge between 2 groups, and also compares adverse events. Statistical significance was set at $p<0.05$ for subjects' characteristic and $p<0.1$ for functional status.

RESULTS

Among 60 ischemic stroke patients, 6 patients were withdrawn from this research: 4 of them due to adverse events, 1 patient died and 1 patient withdrew from the study, there were 54 subjects for complete analysis. Male subjects dominated this study with a total of 36 subjects (60%) compared to 24 female subjects (40%). The subjects' mean age was 61.9±12.462 years old.
The most common comorbid conditions was hypertension in 35 subjects (58.3%). The concomitant medications were 30 (50%) antihypertensive medications, 14 (23.3%) antidiabetic medications, 56 (93.3%) antiplatelet medications and 1 (1.7%) proton pump inhibitor (PPI)/ H2 blocker medications.
Normal powers of muscle strength were present in 3 subjects (5%). Muscle strengths that able to against gravity and resistance, against gravity but not resistance, and make active movement but not against gravity consecutively seen in 23 subjects (38.3%), 20 subjects (33.3%), and 10 subjects (16.7%). Muscle contractions without limbs movement were present in 2 subjects (3.3%), meanwhile there were 2 subjects (3.3%) who did

not show muscle contractions. There was no significant differences in length of stay between experimental and control group (4.44±1.739 vs 3.89±2.276) (p: 0.18).

Table 2 shows significant improvements in mRS (3.30±0.724 vs 1.78±0.974) (p <0.001), NIHSS (7.11±2.806 vs 2.±2.787) (p <0.001) and BI (4.63±3.53 vs 14.52±5.199) (p <0.001) scores after treatment in experimental group. The improvements of mRS (3.11±0.801 vs 2.26±1.059) (p <0.001), NIHSS (6.19±2.788 vs 2.52±3.227) (p <0.001), and BI (6.59±4.693 vs 13.44± 5.905) (p <0.001) in control group were also significant. Differences of mRS (1.52±0.643 vs 0.85±0.534) (p <0.001), NIHSS (5.11±1.826 vs 3.67±1.941) (p: 0.012), and BI (9.96±3.716 vs 6.85±3.146) (p: 0.001) scores between 2 groups were significantly different as written in table 3. Table 4 shows no significance differences of mRS and BI scores at hospital discharge between 2 groups.

Table 5 compares the adverse events between 2 groups. Adverse events which occurred in the experimental group include heartburn, nausea, vomiting, and GI discomfort. Side effects in the control group consisted of dyspepsia, GI discomfort, thrombocytopenia, and decreased hemoglobin. All side effects are at a moderate level. Actions taken include: administration of PPIs, administration of antacids and pantoprazole for heartburn, and transfusions for decreasing hemoglobin. There were no side effects associated with DLBS1033.

Table 1. Subjects' characteristic

	Groups			
	Experimental group	Control group	Total	*p*
Sex				
Male	19	17	36 (60%)	0.598
Female	11	13	24 (40%)	
Comorbid conditions				
Hypertension	18	17	35 (58.3%)	0.793
Diabetes mellitus	10	5	15 (25%)	0.136
Cardiovascular disease	6	4	10 (16.7%)	0.488
Gastrointestinal disease	2	2	4 (6.7%)	1
Others	2	4	6 (10%)	0.671
Concomitant medications				
Antihypertensive medication	15	15	30 (50%)	1
Antidiabetic medication	10	4	14 (23.3%)	0.067
Antiplatelet	28	28	56 (93.3%)	1
PPI/ H2 blocker	1	0	1 (1.67%)	1
Anticoagulant	0	0	0 (0%)	1
Muscle strength				
0 - No contraction	2	0	2 (3.3%)	0.097
1 - Visible muscle contraction without limb movement	2	0	2 (3.3%)	
2 - Active movement, but not against gravity	5	5	10 (16.7%)	
3- Active movement against gravity	10	10	20 (33.3%)	
4 - Active movement against gravity and resistance	11	12	23 (38.3%)	
5 - Normal muscle power	0	3	3 (5%)	
	Experimental group	**Control group**	**Total**	***p***
	Mean (s.d)	**Mean (s.d)**	**Mean (s.d)**	
Age	59.47 (11.506)	64.33 (13.089)	61.9 (12.462)	0.132
Leukocyte counts	9.5283 (3.47443)	9.7315 (3.78016)	9.6359 (0.50476)	0.85
Hemoglobin	14.2083 (1.70496)	13.2115 (2.016)	13.69 (1.92123)	0.065
Hematocrit	41.8625 (5.04534)	39.1 (5.6478)	40.426 (5.4924)	0.074
Platelet counts	277.2083 (87.59193)	307.3462 (222.98645)	292.88 (170.88445)	0.793
Total cholesterol	193.9579 (54.40271)	209.7667 (57.12018)	197.752 (54.28589)	0.566
Low-density lipoproteins	139.325 (56.06603)	135.2167 (46.05199)	138.2045 (52.4761)	0.864
Urea	35.2143 (25.97199)	28.7263 (16.72775)	32.1325 (22.04162)	0.4625
Creatinine	1.141 (0.70392)	1.1237 (0.39968)	1.1328 (0.57263)	0.635
Sodium	139.5619 (4.46872)	140.475 (3.05082)	139.8939 (0.69368)	0.492
Potassium	3.9281 (0.59442)	3.9883 (0.58449)	3.95 (0.58236)	0.64
Random blood glucose	148.5125 (80.997)	155.96 (93.41863)	152.3122 (86.72513)	0.617
Length of stay	4.44 (1.739)	3.89 (2.276)	4.1667 (2.02578)	0.118

Table 2. Improvement of mRS, NIHSS, and BI scores in experimental and control group

	Mean (s.d)	*p*
Improvement of mRS scores in experimental group		
Before therapy	3.30 (0.724)	<0,001
After therapy	1.78 (0.974)	
Improvement of mRS scores in control group		
Before therapy	3.11 (0.801)	<0,001
After therapy	2.26 (1.059)	
Improvement of NIHSS scores in experimental group		
Before therapy	7.11 (2.806)	<0,001
After therapy	2 (2.787)	
Improvement of NIHSS scores in control group		
Before therapy	6.19 (2.788)	<0.001
After therapy	2.52 (3.227)	
Improvement of BI scores in experimental group		
Before therapy	4.63 (3.65)	<0.001
After therapy	14.52 (5.199)	
Improvement of BI scores in control group		
Before therapy	6.59 (4.693)	<0.001
After therapy	13.44 (5.905)	

Table 3. Comparison of differences in mRS, NIHSS, and BI scores between experimental and control group

	Mean (s.d)	*p*
Comparison of the difference in mRS scores between 2 groups		
Difference of mRS scores in experimental group	1.52 (0.643)	<0.001
Difference of mRS scores in control group	0.85 (0.534)	
Comparison of the difference in NIHSS scores between 2 groups		
Difference of NIHSS scores in experimental group	5.11 (1.826)	0.012
Difference of NIHSS scores in control group	3.67 (1.941)	
Comparison of the difference in BI scores between 2 groups		
Difference of BI scores in experimental group	9.96 (3.716)	0.001
Difference of BI scores in control group	6.85 (3.146)	

Table 4. Comparison of mRS and BI scores between experimental and control group at hospital discharge

		mRS scores		*p*
		<2 (good outcomes)	≥2 (poor outcomes)	
Groups	Experimental group	11 (40.7%)	16 (59.3%)	0.387
	Control group	7 (25.9%)	20 (74.1%)	
	Total	18 (33.3%)	36 (66.7%)	
		BI scores		*p*
		≥12 (independent)	<12 (dependent)	
Groups	Experimental group	22 (81.5%)	5 (18.5%)	0.129
	Control group	17 (63%)	10 (37%)	
	Total	39 (72.2%)	15 (27.8%)	

Table 5. Comparison in adverse events between experimental and control group

		Adverse events		*p*
		Yes	No	
Groups	Experimental group	2 (6.9%)	27 (93.1 %)	1
	Control Group	2 (6.9%)	27 (93.1)	
	Total	4 (6.9%)	54 (93.1%)	

DISCUSSION

The main activities of DLBS1033 - fibrinolytic and fibrigenolytic activities - is already known to be a promising therapy for stroke. However, comparison of disability in DLBS1033 therapy and standard therapy has not been much studied, especially with mRS as measuring instrument. This randomized controlled study is aimed to identify the benefit of DLBS1033 as add on therapy in ischemic stroke patients' disabilities and its safety.

This study's subjects were dominated by male with the average of subjects' age was 61.9±12.462 years. This is consistent with previous studies showed that the incidence of stroke in men exceeds the incidence of stroke in women with the average age of 68 years old and a range of 57 - 77 years old; old age increases the risk of stroke [12, 13, 14]

The leading concomitant medications in this study is antiplatelet medication in 56 subjects (93.3%). The mean levels of hemoglobin and hematocrit respectively were 14.2083±1.70496 g/dL and 41.8625±5.04534%. Antiplatelet use, hemoglobin, and hematocrit levels is associated with anemia [15]. Furthermore, anemia decreases the oxygen-carrying capacity in the blood and worsens ischemia [16, 17]. The relationship between hemoglobin and hematocrit with stroke may be U-shaped, because high or low hemoglobin and hematocrit levels are both associated with atherosclerosis and cardiovascular disease [18, 19]

Stroke patients tend to have higher random blood glucose rates than normal random blood glucose rates. The mean of random blood glucose in this study was 148.5125±80.997 mg/dL, therefore this result was above the normal random blood glucose level. The higher random blood glucose rate shows an increased mortality rate [20]. High blood sugar levels will cause vascular endothelial dysfunction, increased early arterial stiffness, systemic inflammation and thickening of the capillary basal membrane [21].

Laboratory results showed that the leukocyte count in this study had a mean of 9.5283±3.47443 thousands/mm^3. An increase in total leukocyte count stimulates a series of reactions by phagocytes which cause damage to blood vessels and develop into atherosclerosis; higher leukocyte count related with poor prognosis and dead [20, 22].

The platelet count in this study had a mean of 277.2083±87.59193/mm^3. Previous study showed only 1.53% of stroke patients had thrombocytosis. Thrombocytosis is associated with ischemic stroke, while thrombocytopenia is associated with hemorrhagic stroke [23].

The mean of total cholesterol and LDL cholesterol in this study was, respectively 193.9579±54.40271 mg/dL and 139.325±56.06603 mg/dL. This is in line with a research in 2018 which found 36.3% of ischemic stroke patients had a history of dyslipidemia, while the remaining 63.7% did not have a history of dyslipidemia [24].

The mean of sodium and potassium level obtained in the study subjects was 139.5619±4.46872 mmol/L and 3.9281±0.59442 mmol/L, these results were still within normal limits. The level of sodium in the blood in hemorrhagic stroke is significantly lower than in ischemic stroke [25]. Hypokalemia is more common in hemorrhagic strokes than ischemic strokes [26].

The mean of urea and creatinine level obtained during the study were 35.2143±25.97199 mg/dL and 1.141±0.70392 mg/dL. Urea and creatinine levels can be used to assess dehydration in stroke. Dehydration reduces total plasma volume and reduces cardiac output thereby contributing to Virchow's Triad in the formation of thrombosis [27]. of sodium and potassium levels that were still within normal limits showed the study subjects had a good prognosis, indicated by significant improvements in mRS, NIHSS, and BI scores in both groups.

There were significant improvements in mRS, NIHSS and BI scores after treatment in experimental and control group. However, The improvement of functional status in the experimental group was better than the control group. The results obtained area similar with the study in China, after 1 year of research there was a reduction in NIHSS scores in the lumbrokinase and control groups with statistically significant differences [28]. Another study in 2016 showed the highest increase in BI scores starting from the beginning of the study until the 90th day was found in the group of patients given DLBS1033. However, the increase in BI scores in the DLBS1033 group did not have a significant difference with the aspirin group or the clopidogrel group [29].

The improvements of functional outcomes were better in experimental group because of these main activities of DLBS1033: fibrinolytic, fibrigenolytic, reduce blood viscosity, and reduce platelet aggregation [5]. Fibrinolytic activity occurs through the conversion of plasminogen to plasmin by extrinsic plasminogen activator or extrinsic plasminogen activator (e-PA). Fibrinolytic activity only works specifically on fibrin, therefore DLBS1033 does not cause excessive bleeding. DLBS1033 does not degrade other plasma proteins, including plasminogen and albumin [30]. Lumbrokinase not only destroys fibrin, but also degrades fibrinogen directly. Meanwhile, aspirin has antithrombotic effects by inhibiting the cyclooxygenase enzyme (COX) and blocking the prostaglandin pathway in platelet activation irreversibly [31]. Thus, aspirin only prevents the formation of new thrombus and blood embolism, but does not destroy the thrombus and blood embolism that has already formed.

The mRS (p: 0.387) and BI (p: 0.129) scores between the treatment and control groups presented in the categorical form did not have significant differences. This is because converting numerical data into categorical data will reduce the precision of research data. In addition, changes in numerical data into categorical data cause misclassification. Various weaknesses from categorical data make numerical analysis more advisable [32].

The limitation of this study was not long enough. This study only observed the functional status in hospital discharge. Nevertheless, this study provides considerable information on benefit and safety of DLBS1033 as add on therapy for ischemic stroke patients.

CONCLUSIONS

DLBS 1033 has proven to improve functional outcomes better than standard therapy. This study did not find any adverse events related to DLBS1033.

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Declaration of Interest

Nothing to declare.

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