ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 1, 2016

ISSN - 0974-2441

Research Article

HEMOSTASIS PROFILE AND CLINICAL OUTCOME OF ACUTE ISCHEMIC STROKE PATIENTS TREATED WITH ORAL LUMBROKINASE DLBS1033: A COMPARATIVE STUDY VERSUS ASPIRIN AND CLOPIDOGREL

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Received: 20 August 2015, Revised and Accepted: 30 September 2015

ABSTRACT

Objectives: This clinical study was conducted to determine the hemostasis profile and clinical outcome of acute ischemic stroke patients treated with DLBS1033 in comparison with aspirin or clopidogrel. DLBS1033 is a proprietary bioactive protein fraction derived from the earthworms (*Lumbricus rubellus*) that possesses both fibrinolytic and antithrombotic properties.

Methods: This was a 3-arm, parallel, randomized, controlled, open-label, blinded-evaluation study involving 126 acute ischemic stroke patients. Each subject received any of the following study medication within 96 hrs after the stroke onset: Aspirin 80 mg daily (Group 1), or clopidogrel 75 mg daily (Group 2), DLBS1033 490 mg 3 times daily (Group 3), for 90 days. Hemostasis parameters evaluated were prothrombin time (PT), activated partial thromboplastin time (aPTT), and international normalized ratio (INR), while the clinical outcomes were measured using Gadjah Mada Stroke Scale (SSGM) and Barthel index (BI).

Results: Baseline characteristics, including the hemostasis and clinical profiles, were comparable between groups. At the end of the study, PT, aPTT, and INR values were not significantly different between groups, which were all within the normal ranges. There was a significant improvement of BI as well as SSGM from baseline in each group. The improvement size of BI was found similar between groups (p=0.098). However, a significantly better improvement of SSGM was observed in those received DLBS1033 (6.98±4.90; p=0.001 vs. aspirin [3.74±3.66], p=0.006 vs. clopidogrel [4.26±4.21]).

Conclusion: It was concluded that DLBS1033 provided a safe hemostasis profile (PT, aPTT, and INR) comparable to that of aspirin or clopidogrel in ischemic stroke patients. Treatment with DLBS1033 improved clinical outcomes indicated by the BI and SSGM, and the improvement size of SSGM was even better than that of aspirin or clopidogrel treatment.

Keywords: Acute ischemic stroke, Barthel index, DLBS1033, Hemostasis, Gadjah Mada Stroke Scale, Oral lumbrokinase.

INTRODUCTION

Thrombosis remains involved in the pathological course of some most common vascular diseases, including ischemic stroke [1,2]. Substantial progress has been made in understanding the biology of thrombus formation and the pathophysiology of thrombosis. Several more established pharmacologic agents, including thrombolytic therapy, antiplatelet agents, and anticoagulants, have been recommended for the early management of acute ischemic stroke [3]. However, all the recommended, as well as neuro-protective agents available for prevention or treatment that have been in use for decades, have currently been replaced with newer variants that offer a modest incremental improvement [4-10]. Yet, the ideal drug for prophylaxis and treatment of thrombotic disease that will inhibit the thrombosis but not the hemostasis remains scarce. This situation is complicated further by the emerging resistance to therapy with the most established antiplatelets, aspirin, and clopidogrel that brings potentially severe consequences such as recurrent myocardial infarction, stroke, or death [11-13]. Such a reduced sensitivity to antiplatelet drugs was even reported to be more remarkable in diabetic as compared to non-diabetic patients [14]. Thus, an expedite translation of new knowledge from "test-tube," and animal studies to bedside pharmaceutical development should be pursued for new and more strategic advances in the prevention of thrombotic diseases.

For thousands of years, earthworms have widely been used in Indonesia, China, Japan, and the Far East to treat various chronic diseases. A group of serine protease enzymes collectively called lumbrokinase extracted from the earthworms of Lumbricidae family could directly dissolve fibrin and activate plasminogen [15,16]. Lumbrokinase possesses strong fibrinolytic and fibrinogenolytic properties, lowers blood viscosity, markedly inhibits platelet aggregation, and promotes thrombus degradation without causing excessive bleeding [17-19]. Lumbrokinase is stable over a wide range of pH and temperature; thus, it can be administered orally [20].

DLBS1033 investigated in this clinical study is a lumbrokinase fractionated from the earthworms, *Lumbricus rubellus*, through a proprietary technology of extraction. DLBS1033 possesses eight major proteins, each with a molecular weight below 100 kDa, named as Lumbricus low-molecular-weight proteins [21]. This specific pattern of proteins confers a unique characteristic of DLBS1033 with its mechanism of action as an antithrombotic and thrombolytic agent. The antithrombotic and thrombolytic activities of the bioactive protein fraction have been demonstrated *in vitro* and *ex vivo* [21]. Furthermore, DLBS1033 has also been proven for its safety profile, through toxicological studies in animal [22], and safety studies in human [23,24]. To date, DLBS1033 has been approved by National Agency of Drug and Food Control, Republic of Indonesia, to be marketed as Indonesian standardized herbal medicine. Since then, no clinically significant adverse drug reactions have been reported.

This current study was conducted to investigate whether the virtues of DLBS1033 that have been demonstrated preclinically would also be translated into clinical benefits for ischemic stroke patients. In this study, we evaluated the hemostasis profile as well as functional and neurological outcomes of ischemic stroke patients receiving DLBS1033, in comparison with the more established antiplatelet agents used in such cases, aspirin and clopidogrel.

METHODS

Study design

This study was conducted in compliance with the Declaration of Helsinki and the Good Clinical Practice. The study protocol was reviewed and approved by the Independent Ethics Committee of Gadjah Mada University, Yogyakarta, Indonesia, prior to trial initiation. This was a 3-arm, parallel, randomized, controlled, open-label, blinded-evaluation (PROBE-designed) clinical study, over 90 days of treatment, to determine the hemostasis profile as well as functional and neurological outcomes of ischemic stroke patients. Eligible subjects were randomly allocated to receive any of the following regimens: Aspirin 80 mg once daily (Group 1, Aspirin), clopidogrel 75 mg once daily (Group 2, Clopidogrel), or DLBS1033 490 mg three times daily (Group 3, DLBS1033). In this study, both aspirin and clopidogrel served as the positive controls to examine the efficacy and safety of the new treatment with DLBS1033.

Subjects were acute ischemic stroke patients admitted in the Stroke Unit or Neurology-Ward of the Central General Hospital Dr. Sardjito Yogyakarta, Indonesia. The inclusion criteria included: (1) adult male or female; (2) acute ischemic stroke diagnosed by cranial CT-scan; (3) having admitted in the hospital within less than 96 hrs after the onset of stroke; and (4) willingness to participate in the study and give subject's written informed consent. The patients were excluded if any of the following criteria was met: (1) recurrent stroke; (2) transient ischemic attack; (3) intra-cerebral or subarachnoid hemorrhagic stroke; (4) undefinable stroke onset; (5) regular therapy with antiplatelets, anticoagulants; (6) hemostasis or coagulation disorders; (7) major surgery within the last 6 months or having to have a surgery within the next 3 months; (8) renal impairment defined as serum creatinine level >3× upper limit of normal or history of hemodialysis; (9) systemic inflammatory response syndrome; (10) unconsciousness; or (11) uncontrolled hypertension (systolic blood pressure > 185 mmHg or diastolic blood pressure > 110 mmHg).

Subjects were withdrawn from the study if any of the following conditions applied during the course of the subject's participation: (1) Worsened prognosis, unconsciousness, and neurological deficits; (2) hypersensitive to any of the study medication; (3) bleeding adverse events, including nausea and vomiting, severe headache, gastrointestinal pain, hematoma, and any of bleeding signs; and (3) subjects were suffering from any disease, including physical accidents that would interfere with the evaluation of the hemostasis profile.

Study medication

The management of acute ischemic stroke in the Unit Stroke of Dr. Sardjito Yogyakarta Hospital was in accordance with the acquired hemophilia A (AHA)/ASA Guidelines for the Early The Management of Patients with acute ischemic stroke.[3] Aspirin (Thrombo Aspilets® enteric-coated tablets, Medifarma Laboratories Inc., Jakarta, Indonesia) at the dose of 80 mg once daily, clopidogrel (Vaclo® film-coated tablets, Dexa Medica, Palembang, Indonesia) 75 mg [25], once daily, or DLBS1033 490 mg (Disolf® enteric-coated tablets, Dexa Medica, Palembang, Indonesia) three times daily was initially administered to the eligible subjects within 96 hrs after the stroke onset, to be taken for the next 90 days. Concomitant medications taken by study subjects along the study were valsartan 80/160 mg daily and/or amlodipin 5/10 mg daily (by hypertensive subjects); (2) insulin therapy (by diabetic subjects); (3) simvastatin 20 mg daily (all subjects); and (4) oral citicholine 1000 mg twice daily (all subjects). After study completion, subjects continued their therapy in accordance with the management for ischemic stroke applicable in the hospital.

Characterization of DLBS1033 was as previously described by Trisina *et al*. [21]. A pharmaceutical study has also been conducted to support its oral enteric-coated formulation and dosing regimen [26].

Randomization code was prepared using the permuted 3-block-allocation and random numbers generator. In order to keep the blinded-evaluation procedures, the study products were administered by a particularly designated nurse, and the Investigator who evaluated the outcomes was blind of the subject allocation. Blinding codes were disclosed when the study had been completed, and trial-related database had been frozen.

Hemostasis profile and clinical outcomes

Hemostasis parameters evaluated in this study were prothrombin time (PT), activated partial thromboplastin time (aPTT), and international normalized ratio (INR). Adverse events were observed and recorded during the study conduct. The aPTT is an appropriate parameter to evaluate the intrinsic and common pathways of the coagulation cascade. The PT (or can be expressed as INR) evaluates the extrinsic and common pathways [27]. Therefore, measurement of PT (or INR), or aPTT can be beneficial to detect the abnormal steps in the coagulation cascade, for the diagnosis of coagulation disorders [28]. Since we expected that therapy with DLBS1033 as an antithrombotic/thrombolytic agent should not impair the hemostasis profile, we hypothesized that the bioactive fraction would not significantly interfere with those coagulation parameters.

The functional outcomes evaluated in this study were Barthel index (BI) [29-33]. All variables were measured at baseline (day 0, before study medication) and day 90 (end of study). The BI is based on a rating scale that is completed by an observer, and it has a good inter-rater reliability [30]. The items can be divided into a group that is related to self-care (feeding, grooming, bathing, dressing, bowel and bladder care, and toilet use) and a group related to mobility (ambulation, transfers, and stair climbing). A total of 10 activities are scored, and the values are then added to give a total score ranging from 0 (totally dependent) to 100 (completely independent). Lower scores indicate greater dependency. Scoring on the BI can be interpreted as follows: Independent (BI score of 80-100), needs minimal help (60-79), partially dependent (40-59), very dependent (20-39), and totally dependent (<20). The BI measures what the patient actually does rather than what they can do [30-32].

Neurological outcome in this study was measured by the validated Gadjah Mada Stroke Scale (SSGM) [34]. The SSGM consists of 14 items, including level of consciousness, orientation, language, visual field, eye movement, facial movement, motoric function of the arms and limbs both the passive and active (affected) sides, each of which is scored 0 (severe impairment) to 2 or 3 (normal, without impairment), resulting in a total score ranging from 0 (the most severe neurological deficit) to 38 (complete recovery) as shown in Table 1. The interpretation of the SSGM is that a score of ≥23 means mild to moderate neurological deficits, and a score of <23 means severe neurological deficits [34].

Statistical analysis

Demography and baseline characteristics were tabulated and summarized by the group. Analyzes on hemostatic parameters as well as clinical outcomes were carried out on intent-to-treat (ITT) population, consisting of all patients who were randomized, exposed to at least one dose of the study product, and then returned for at least one visit after treatment-initiation. This would include data from withdrawn subjects.

All of the data are expressed as mean±standard deviation unless otherwise is specified. Comparability of baseline characteristics between groups was statistically analyzed by Kruskal–Wallis and Chi-square, for continuous and categorical data, respectively. Hemostasis and efficacy parameters (PT, aPTT, INR, SSGM, and BI) were statistically analyzed between groups using Kruskal–Wallis and within-group using Wilcoxon signed-rank. All statistical tests were at 5% significance level. The SPSS® version 14.0 was used for the analyzes.

RESULTS

The clinical study had been conducted in the Stroke Unit or Neurology-Ward of the Central General Hospital Dr. Sardjito Yogyakarta since May 2012 until December 2013. A total of 129 subjects were enrolled and randomly allocated into any of the 3 arms, each of which consisted of 43 subjects. Of 129 subjects, only 126 were available for ITT analysis.

Table 1: SSGM [34]

Tested item	Responses and scores
Level of consciousness	3 - Alert 2 - Drowsy, somnolent 1 - Obtunded, stupor 0 - Coma/unresponsive
Orientation (time, space, people) questions	3 - Answers each of all three questions correctly 2 - Answers two of three questions correctly 1 - Answers one of three questions correctly 0 - Answers neither correctly
Articulation	3 - Normal 2 - Mild dysarthria 1 - Severe dysarthria 0 - Mute or global aphasia
Gaze	3 - Normal horizontal movements 2 - Eyeball in medial position, able to deviate to either side 1 - Eyeball in lateral position, able to return to medial position 0 - Complete gaze palsy (conjugate deviation)
Facial movement	2 - Normal 1 - Partial facial weakness (paresis) 0 - Complete unilateral palsy (paralysis)
Visual fields	2 - Normal 1 - Partial hemanopia 0 - Complete hemanopia
Arm's motoric function - Passive (unaffected side) Examiner raises both subject's arms to a position of 45° (if the subject is in a supine position), or 90° (if seated). Subject is asked to hold such a position for 10 seconds	3 - No drift 2 - Falls before10 seconds 1 - Unable to hold, but performs noticeable effort against gravity 0 - No effort against gravity
Arm's motor function - Active (affected side) Examiner raises both subject's arms to a position of 45° (if the subject is in a supine position), or 90° (if seated). Subject is asked to hold such a position for 10 seconds	3 - Able to raise perfectly 2 - Able to raise 1 - Able to raise with flexed arm 0 - No movement
Extension of wrist	3 - Full extension, full strength 2 - Full extension, less strength 1 - Partial extension 0 - Unable to extend
Fingers strength Subject is asked to perform pinch (squeeze between the finger and thumb) with both hands, and the examiner has to release the squeeze with one finger	2 - Balanced strength of both hands 1- Less strength on the affected hand 0 - Unable to perform pinching
Limb's motoric function - Passive (unaffected side) Examiner raises both subject's limbs to a position of 30° (with the subject in a supine position). Subject is asked to hold such a position for 5 seconds	3 - No drift 2 - Falls before 5 seconds 1 - Unable to hold, but performs noticeable effort against gravity 0 - No effort against gravity
Limb's motoric function - Active (affected side) Subject is asked to perform thigh and knee flexion	2 - Full flexion 1 - Partial flexion 0 - No flexion
Dorsiflexion	2 - Normal strength to perform dorsiflexion 1 - Less strength to perform dorsiflexion 0 - Unable to perform dorsiflexion
Gait	4 - Walks 5 m long without aid or other person's assistance 3 - Walks with aid (no assistance from other person) 2 - Walks with assistance from other person 1 - Unable to walk, but able to stand with aid 0 - Neither walks or stands

SSGM: Gadjah Mada Stroke Scale

Three subjects (one subject in each group) moved out of town and were lost to follow-up. They did not have any post-treatment data, thus were not evaluable.

Baseline characteristics of the study subjects were comparable between groups as shown in Table 2. In all groups, subjects were aged between 50 and 65 years old, with male predominance (around 70% of subjects in each group).

Onset of stroke, BI, PT, aPTT, and INR, as well as other risk factors, such as plasma glucose, lipid profile, blood pressure, were also comparable between groups at baseline. In terms of SSGM, at baseline, subjects in DLBS1033 Group showed a slightly lower (worse) score (p=0.030) than those in the aspirin group. Therefore, the real between-group difference in neurological outcome was also evaluated by comparing the size of improvement of SSGM from baseline, in addition to the final score of SSGM at the end of study.

Table 2: Baseline characteristics

Variable	Group 1 Aspirin (n=42)	Group 2 Clopidogrel (n=42)	Group 3 DLBS1033 (n=42)	p‡
Age (year)	58.81±9.40	62.45±10.54	61.29±8.73	0.210
Gender (%)				
Male	31 (73.8)	29 (69.0)	31 (73.%)	0.854
Female	11 (26.2)	13 (31.0)	11 (26.2)	
Onset of stroke (hours before initial dose of study medication)	25.26±32.85	23.24±26.75	33.52±47.41	0.399
Blood pressure (mmHg)				
Systolic	166.90±35.97	162.74±26.09	168.45±29.39	0.570
Diastolic	98.69±18.45	93.93±11.77	95.60±13.58	0.356
Concomitant diseases (%)				
Hypertension	22 (52.4)	15 (35.7)	21 (50.0)	0.253
Diabetes mellitus	18 (42.9)	14 (33.3)	19 (45.2)	0.501
Dyslipidemia	32 (76.2)	24 (57.1)	20 (47.6)	0.024*
Concomitant medication (%)				
Amlodipine	15 (35.7)	10 (23.8)	13 (31.0)	0.489
Valsartan	11 (26.2)	6 (14.3)	11 (26.2)	0.317
Insulin	18 (42.9)	14 (33.3)	19 (45.2)	0.501
Clinical outcomes				
Barthel index	81.43±23.07	73.57±28.14	71.55±27.42	0.262
SSGM	32.05±5.66	29.64±7.37	28.52±6.67	0.030*
Laboratoric parameters				
FPG (mg/dl)	143.36±68.44	144.36±80.23	141.74±69.03	0.927
Hemoglobin (g/dl)	14.58±1.52	13.88±1.76	14.28±1.57	0.365
Total cholesterol (mg/dl)	226.21±43.84	217.69±48.02	214.28±45.61	0.222
HDL (mg/dl)	42.81±9.36	43.71±11.96	44.41±9.99	0.876
LDL (mg/dl)	160.08±36.74	140.79±35.63	142.24±32.03	0.044*
Triglyceride (mg/dl)	173.40±90.83	154.17±80.92	147.27±61.66	0.559
BUN (mg/dl)	13.10±3.93	12.55±3.18	12.80±3.07	0.908
Serum creatinin (mg/dl)	1.23±0.70	1.17±0.41	1.09±0.51	0.766
Uric acid (mg/dl)	6.48±1.42	6.20±1.72	6.50±1.50	0.748
PT (seconds)	12.98±1.22	13.07±1.15	13.09±1.29	0.866
aPTT (seconds)	29.66±1.22	29.39±3.39	29.65±4.73	0.978
INR	0.94±0.16	0.93±0.13	0.97±0.15	0.545

Continuous data are expressed in mean±SD. Categorical data are expressed in a number of subjects (n) and percentage (%). ‡Between-group analysis: Categorical variables were analyzed using Chi-square; while the continuous ones using Kruskal–Wallis. aPTT: Activated partial thromboplastin time, BUN: Blood urea nitrogen, FPG: Fasting plasma glucose, HDL: High-density lipoprotein cholesterol, INR: International normalized ratio, LDL: Low-density lipoprotein cholesterol, PT: Prothrombin time, SSGM: Gadjah Mada Stroke Scale, SD: Standard deviation

After 90 days of treatment, we found no difference in hemostasis profile between groups as shown by PT, aPTT, and INR values (Table 3). Within-group analysis showed that there was a bit of shortened PT with DLBS1033, but of no clinical importance. The aPTT and INR in each group did not change from their respective baseline values. All measured hemostasis parameters in each group remained within their respective normal ranges.

Hemostasis profile at the end of the study demonstrated that administration of DLBS1033 at the dose of 3 × 490 mg daily for 90 days in ischemic stroke subjects was safe and comparable to aspirin 80 mg daily or clopidogrel 75 mg daily. There was no significant prolongation of hemostasis parameters found. Neither were there bleeding adverse events in any groups observed during the study conduct.

All subjects in all groups received simvastatin 20 mg daily and citicholine 1000 mg twice daily during the study participation. For the antihypertensive agents, subjects might receive amlodipine, valsartan or a combination of both, depending on their individual condition. All diabetic subjects received insulin therapy.

In terms of clinical outcome, each group demonstrated a significant improvement of BI (p<0.001) from baseline to day 90, with a mean score of >85 in all groups at the end of study. The greatest improvement was observed in DLBS1033 Group, with the size of improvement of 23.09±19.16 from baseline, but it was not significantly different (p=0.098) with that of aspirin (15.12±15.71) or clopidogrel (17.98±19.03), as shown in Fig. 1.

In line with the improvement of BI, DLBS1033 Group demonstrated the greatest improvement of SSGM (6.98±4.90) from baseline that was also significantly greater (p=0.002) than that of aspirin (3.74±3.66) or Clopidogrel (4.26±4.21) (Fig. 2).

Table 3: Hemostasis profile between groups at baseline and day 90

Hemostasis profile	Baseline	p‡	Day 90	p‡	p†
PT (second)					
Aspirin (n=42)	12.98±1.22	0.866	12.36±1.39	0.788	0.080
Clopidogrel (n=42)	13.07±1.15		12.53±1.19		0.076
DLBS1033 (n=42)	13.09±1.29		12.67±1.55		0.039*
aPTT (second)					
Aspirin (n=42)	29.66±5.49	0.978	29.59±4.25	0.619	0.523
Clopidogrel (n=42)	29.39±3.39		29.96±4.16		0.302
DLBS1033 (n=42)	29.65±4.73		30.54±4.41		0.096
INR					
Aspirin (n=42)	0.94±0.16	0.545	0.94±0.13	0.154	0.318
Clopidogrel (n=42)	0.93±0.13		0.96±0.11		0.051
DLBS1033 (n=42)	0.97±0.15		0.99±0.13		0.132

Data are expressed in mean±SD. †Within-group analysis using Wilcoxon Signed-Rank. ‡Between-group analysis using Kruskal–Wallis. *Statistically significant (p<0.05). aPTT: Activated partial thromboplastin time, INR: International normalized ratio, PT: Prothrombin time, SSGM: Gadjah Mada Stroke Scale, SD: Standard deviation

We also found there were similar percentages of subject in DLBS1033 Group (83.3% and 100%) to those in aspirin (92.9% and 100%) who achieved BI of ≥85 and SSGM of ≥23, respectively, at the end of study; both of which were slightly higher than those in clopidogrel (73.8% and 97.6%, respectively), even though they were not statistically different (Fig. 3). Compared to aspirin, treatment with DLBS1033 for 90 days seemed to be effectively comparable in achieving BI ≥85 (odds ratio

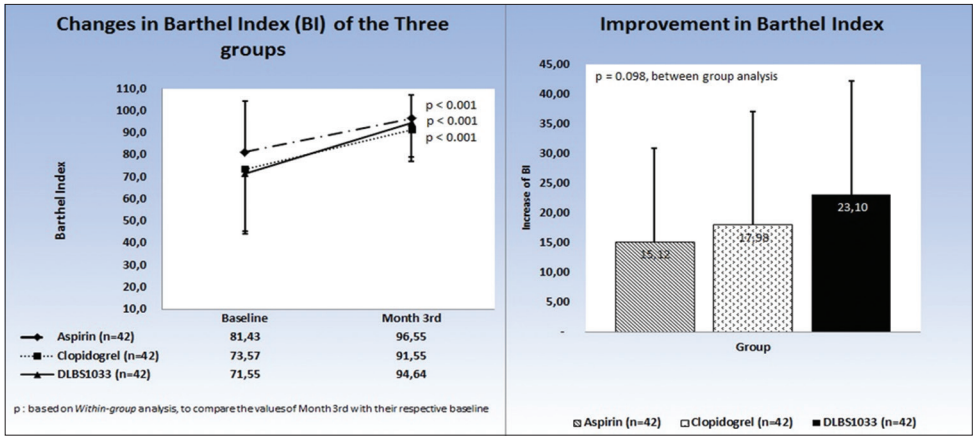

Fig. 1: Effect of aspirin, clopidogrel, and DLBS1033 treatment on the functional outcome measured by Barthel index. The error bars represent the standard errors; Wilcoxon signed-rank was used in within-group analysis; Kruskal–Wallis was used in between-group analysis

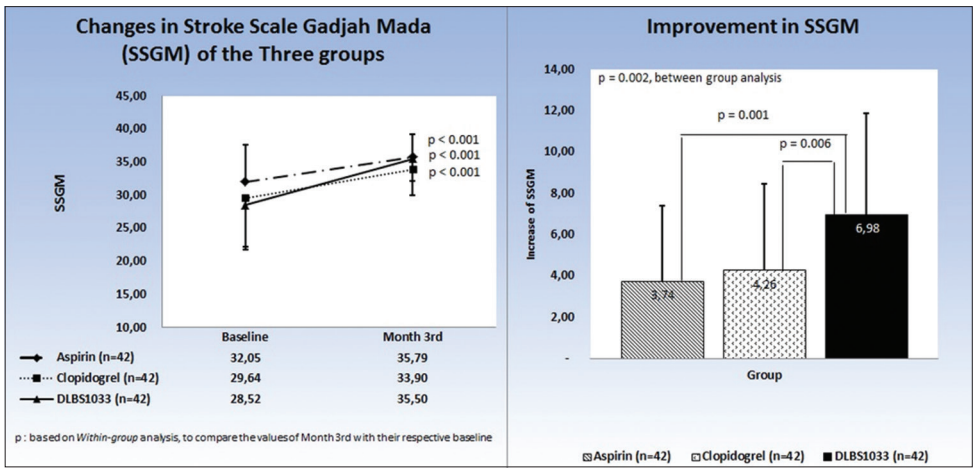

Fig. 2. Effect of aspirin, clopidogrel, and DLBS1033 treatment on the neurological outcome measured by Gadjah Mada Stroke Scale. The error bars represent the standard errors; Wilcoxon signed-rank was used in within-group analysis; Kruskal–Wallis was used in between-group analysis

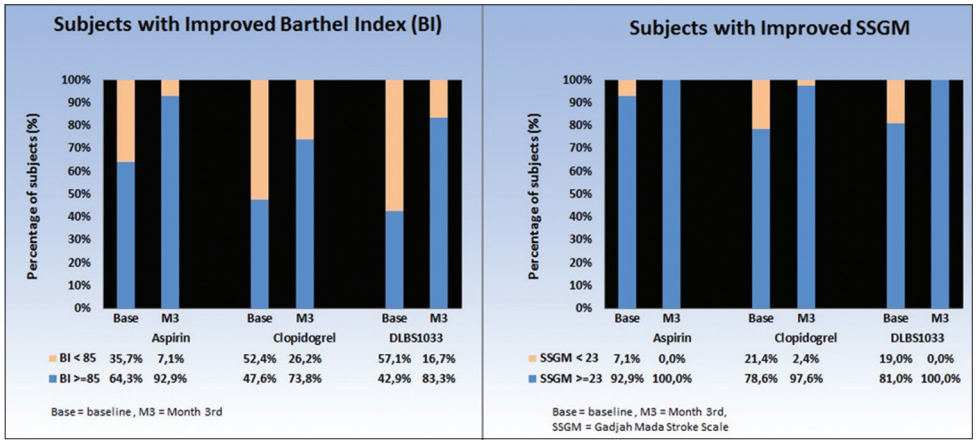

Fig. 3: Proportion of subjects with Bathel index ≥85 (left) and Gadjah Mada Stroke Scale ≥23 (right)

[OR]=0.38; 95% confidence interval [CI], 0.09-1.60; p=0.189). That was also the case with DLBS1033 in comparison with clopidogrel (OR= 1.77; 95% CI, 0.61-5.14; p=0.291). DLBS1033 treatment was also effectively comparable in achieving SSGM ≥23, either compared to aspirin (OR=1.00; 95% CI, 0.019-51.57; p=1.000) or clopidogrel (OR=3.07; 95% CI, 0.12-77.60; p=0.496).

DISCUSSION

This study demonstrated that DLBS1033 therapy in ischemic stroke patients provided a safe hemostatic profile, which was comparable to that of aspirin or clopidogrel therapy (Table 3). All measured parameters, i.e., PT, aPTT, and INR, were within the normal range at the

end of the study, suggesting that there were no deficient coagulation factors observed during therapy with DLBS1033, aspirin, and clopidogrel. Furthermore, the result of this study also indicates that the risk for bleeding due to the administration of DLBS1033 was evidently low, similar to that of aspirin or clopidogrel at their respective usual dose regimen. This was also clinically proven by zero bleeding event in all groups observed during the study conduct. The safety profile of DLBS1033 was aligned to that reported by Zhang in a former study with a similar protease enzymes, lumbrokinase [20], demonstrating that the proteases did not affect hemostasis profile measured by PT or aPTT, thus did not interfere with the INR.

With respect to aspirin and clopidogrel treatment, our current 3-month-study showed no prolongation of aPTT. Neither did it show the increase of INR nor any bleeding events. This was somewhat different with a former report by Tamura *et al*, in which aspirin and clopidogrel treatment were reported associated with bleeding complications (hemorrhage, melaena, and hematochezia). Aspirin was also reported associated with prolongation of aPTT, but not with increased INR. While clopidogrel treatment was associated neither with prolongation of aPTT nor increased INR [35]. However, in the report, aspirin might be used concomitantly with warfarin and/or clopidgrel and for a long-term therapy as well, while the observation in our study was limited only to the first 3 months of therapy. That may explain the difference with the former report. In this study, we found neither clinically significant bleeding events nor adverse changes of the laboratory hemostatic parameters. A case study also reported that clopidogrel treatment was associated with prolonged aPTT and induced AHA in a patient with cerebellar infarction, but the mechanism of how the drug could induce the event is unknown [36], and thus, the adverse effect was likely to be an anecdotal case.

In addition to its promisingly safe hemostatic profile, DLBS1033 also improved the clinical outcomes. A significant improvement of BI from baseline occurred in each treatment group. The improvement was similar across all groups (Fig. 2). This result suggested that DLBS1033 was as efficacious as aspirin or clopidogrel in improving functional outcome of patients with ischemic stroke. This study also demonstrated that DLBS1033 treatment was effectively comparable to aspirin or clopidogrel in achieving BI of ≥85 that means DLBS1033 treatment effectively improved subjects' daily performance from disability or necessity for other people's assistance to becoming independence. A score of 85 of BI usually corresponded to independence with minimal assistance that the majority of patients were able to get dressed and to move from armchair to bed without assistance [37]. Score of <85 also corresponded to a dependent state in which patients reported needing assistance in performing daily living activities, with a sensitivity of 94-95% and a specificity of 80-86% [38-40].

Further, even though that the efficacy of DLBS1033 treatment seemed to be comparable to aspirin or clopidogrel in achieving SSGM of ≥23, the size of improvement of the neurological function was significantly greater with DLBS1033 than that with aspirin or clopidogrel (Fig. 2).

In this study, the improvement of both functional and neurological outcome with DLBS1033 treatment was likely due to DLBS1033 activities as both the antithrombotic and thrombolytic agent [21,41]. *In vitro* studies demonstrated reduced expression of several genes involved in inflammatory and atherogenic reaction by DLBS1033, such as nuclear factor kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), vascular cell adhesion molecule-1 (VCAM-1), and P-selectin. The down-regulation of NF-κB has a link to the inhibition of atheroma formation, suggesting that DLBS1033 works as an anti-atherogenesis agent. DLBS1033 can inhibit the progression of other cytokines that are activated by TNF-α and adherence of leukocytes to endothelium. Activity of DLBS1033 in decreasing the expression of P-selectin may be related to the suppression of *de novo* synthesis of P-selectin mediated by cytokine. DLBS1033 suppressed the expression of VCAM-1, a member of the immunoglobulin gene superfamily that mediates leukocyte binding to the endothelial cell. In the same study, it was shown that DLBS1033 suppressed the expression of MMP-9 gene, a marker of plaque instability, suggesting that this bioactive protein fraction has the ability to control plaque stabilization. MMP-9 is a protease that degrades extracellular matrix proteins including gelatin, collagen, elastin, and laminin that are important in tissue destruction; and also in tissue remodeling and inflammation. This study indicated that DLBS1033 could regulate the uncontrolled event of plaque rupture by inhibiting the expression of MMP-9 [21,41]. Our current clinical findings indicate that DLBS1033 is promising in accommodating the necessity of treatment for acute ischemic diseases, due to its dual features Asan antiplatelet and thrombolytic agent and the oral formulation offers more practical administration.

Due to the limited scope of our study, we did not discuss to what extent other variables such as age, NIHSS score at admission, cardio-/cerebro-vascular history (such as myocardial infarction and stroke), dementia, socio-economic status, presence of fever, undernutrition, as were also reported in former studies [42-47], might influence the outcomes. However, random allocation applied in this study allowed a comparable distribution of those confounding factors between groups; therefore, a valid and reliable interpretation could still be made as previously discussed.

CONCLUSIONS

The study concluded that treatment with DLBS1033 at the dose of 490 mg 3 times daily for 90 days in ischemic stroke subjects demonstrated a safe hemostatic profile, which was comparable to that of aspirin 80 mg once daily or clopidogrel 75 mg once daily. In terms of functional and neurological outcomes, the study indicated that DLBS1033 clinically benefitted the ischemic stroke subjects.

ACKNOWLEDGMENTS

The study was supported by Dexa Medica, Indonesia. The technical assistance of Liana W. Susanto, MBiomed., in the statistical analysis of the data as well as manuscript preparation is gratefully appreciated.

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