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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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 26 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 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 Lumbirubin 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 were met: (1) recurrent stroke; (2) transient ischemic attack; (3) intra-cerebral or subarachnoid hemorrhagic stroke; (4) undefined 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, hematemesis, 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. Aspirin (Thrombo Aspillets® enteric-coated tablets, Medifarma Laboratories Inc., Jakarta, Indonesia) at the dose of 80 mg once daily, clopidogrel (Vacio® film-coated tablets, Dasa Medica, Palembang, Indonesia) 75 mg daily, or DLBS1033 490 mg (Disol® enteric-coated tablets, Dasa 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 were summarized in Table 1. 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 post-test analysis by Bonferroni. All statistical tests were at 5% significance level. The SPSS® version 14.0 was used for the analyses.

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.
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).

Table 1: SSGM [34]

<table>
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<tr>
<th>Tested item</th>
<th>Responses and scores</th>
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| 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) |
| Visual fields | 2 - Normal  
1 - Partial hemianopia  
0 - Complete hemianopia |
| Arm’s motoric function - Passive (unaffected side) | 3 - No drift  
2 - Falls before 10 seconds |
| Arm’s motoric function - Active (affected side) | 1 - Able to raise perfectly  
2 - Able to raise |
| Extension of wrist | 3 - Full extension, full strength  
2 - Full extension, less strength  
1 - Partial extension  
0 - Unable to extend |
| Fingers strength | 2 - Balanced strength of both hands  
1 - Less strength on the affected hand |
| Limb’s motoric function - Passive (unaffected side) | 3 - No drift  
2 - Falls before 5 seconds |
| Limb’s motoric function - Active (affected side) | 1 - Able to hold, but performs noticeable effort against gravity  
0 - No effort against gravity |
| Dorsiflexion | 2 - Full flexion  
1 - Partial flexion  
0 - No flexion |
| 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

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.
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).

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 189
(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, lambrokinase [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 hematocoezia). 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 clopidogrel 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 ≥23 that means the treatment effectivity of 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 atheregenic 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-/cerebrovascular 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 benefited the ischemic stroke subjects.

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