A Clinical Trial on Biological Half Life of Bioactive Protein from *Lumbricus rubellus*, DLBS1033 in Healthy Volunteers

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ABSTRAK

Latar belakang: *DLBS1033 adalah fraksi protein bioaktif yang diekstraksi dari Lumbricus rubellus, dan dari studi in vitro diketahui memiliki aktivitas fibrinogenolitik, fibrinolitik dan antiagregasi. Waktu paruh obat dalam plasma merupakan parameter yang penting dalam menghitung dosis obat. Studi ini dilakukan untuk mengevaluasi waktu paruh biologis DLBS1033 melalui pengukuran kadar plasmin-antiplasmin complex (PAP complex). PAP complex adalah senyawa hasil proses fibrinolisis yang stabil dan inaktif.* ***Metode:*** *desain studi ini adalah uji klinik terbuka pada subyek dewasa sehat. Subyek dibagi menjadi dua kelompok, kelompok yang mendapatkan dosis tunggal (diberi obat 3 x 490 mg) dan kelompok yang mendapatkan dosis berulang hingga mencapai steady state (diberi obat 3 x 490 mg/hari selama 3 hari). Sampel darah untuk pemeriksaan konsentrasi PAP complex diambil pada jam ke-0 (sebelum pemberian obat pada kelompok dosis tunggal), jam ke-0,5, 1, 1,5, 2, 3, 4, 6, 8, 10, 12, dan 24. Parameter keamanan yang diperiksa pada penelitian ini adalah kreatinin, prothrombin time (PT), activated partial thromboplastin time (aPTT), SGOT, dan SGPT.* ***Hasil:*** *waktu paruh biologis DLBS1033 dihitung berdasarkan rerata kadar PAP complex pada tiap waktu pengambilan sampel darah di tiap kelompok. Pada kelompok dosis tunggal, rerata tertinggi konsentrasi PAP complex tercapai sebelum pemberian obat. Hasil ini menunjukkan bahwa aktivitas DLBS1033 tidak bermakna ketika diberikan sebagai dosis tunggal. Pada keadaan steady state, konsentrasi PAP complex meningkat dalam 2 jam setelah pemberian obat terakhir. Waktu paruh biologis DLBS1033 adalah 8,6 jam. Pada penelitian ini tidak didapatkan hasil laboratorium yang bermakna dan kejadian tidak diinginkan yang serius.* ***Kesimpulan:*** *pada penelitian ini disimpulkan bahwa efek fibrinolitik DLBS1033 dapat diukur pada keadaan steady state. Waktu paruh biologis DLBS1033 pada kelompok steady state adalah 8,6 jam. Tidak ditemukan kejadian tidak diinginkan yang serius pada kedua kelompok subyek.*

Kata kunci: *Lumbricus rubellus, fibrinolitik, plasmin-antiplasmin complex, waktu paruh biologis, DLBS1033.*

ABSTRACT

Background: *DLBS1033 is a bioactive protein fraction extracted from Lumbricus rubellus, with fibrinogenolytic, fibrinolytic and anti-aggregation activities reported in an in vitro study. Plasma half-life is an important parameter to calculate its dose. This study was conducted to evaluate the biological half-life of DLBS1033 by measuring serial plasmin-antiplasmin (PAP) complex. PAP complex is a stable and inactive compound as a result of fibrinolysis process.* ***Methods:*** *this was an open-label clinical trial in healthy adult subjects. Subjects were divided into two*

groups to receive single dose drugs (received 3 x 490 mg) or repeated administration until steady state conditions (3 x 490 mg/day for 3 days). Blood samples for PAP complex measurement were collected at time 0 (before drug administration for single dose group), then at 0.5, 1, 1.5, 2, 3, 6, 8, 10, 12, and 24 hours after drug administration. Safety parameters used in this study were creatinine, prothrombin time (PT), activated partial thromboplastin time (aPTT), SGOT, and SGPT. ***Results:*** *the biological half-life of DLBS1033 was calculated based on the mean of PAP complex concentration on each time sampling. In single dose group, the highest mean of PAP complex concentration was reached before drug administration. Our result showed that the activity of DLBS1033 could not be determined after single dose administration. In steady state condition, the PAP complex concentration increase in 2 hours after last drug administration. The biological half-life of DLBS1033 was 8.6 hours. There were no significant safety findings on all laboratory parameters and no serious adverse events.* ***Conclusion:*** *it is concluded that the fibrinolytic effects of DLBS1033 can be measured in steady state condition. The biological half-life of DLBS1033 in steady state condition was 8.6 hours. There were no serious adverse events on two groups of subjects.*

Keywords: *Lumbricus rubellus, fibrinolytic, plasmin-antiplasmin complex, biological half-life, DLBS1033.*

INTRODUCTION

Enzyme from earthworm alimentary tract has been known for its ability to dissolve fibrin. In 1991, Mihara et al[1] extracted enzyme from alimentary tract of Lumbricus rubellus, which consist of six isoenzyme serine protease that collectively named as lumbrokinase.[1] From several studies, it has been known that lumbrokinase has fibrinolytic, fibrinogenolytic, antiinflammatory activities, and could reduce platelet aggregation.[2-4]

The efficacy of oral lumbrokinase has been investigated in some clinical trials. Jin et al[5] conducted a study on 51 cerebral infarct subjects who was given oral lumbrokinase for 28 days. They concluded that mechanism of lumbrokinase are inhibit intrinsic coagulation pathway and activate fibrinolytic pathway by increasing t-PA activity.[5] Fibrinolytic activity was also concluded by Rey[6], who conducted a study on 28 diabetic foot ulcer subjects, which were given three times 500 mg lumbrokinase/placebo per day (n=14) for seven days. In the treatment group, D-dimer was increased.

From many clinical trials, it is concluded that effects of lumbrokinase can be seen after several days. It is different with intravenous fibrinolytic enzyme which effects can be seen immediately after used. Therefore oral lumbrokinase could not replace the function of an intravenous fibrinolytic enzyme which is used on acute thrombosis. Oral lumbrokinase might be used for secondary prevention after acute thrombosis, such as myocardial infarct and stroke.

DLBS1033 is bioactive protein fraction which is extracted from *Lumbricus rubellus* earthworm. This earthworm comes from Pengalengan, West Java, Indonesia. DLBS1033 possesses eight major proteins with molecular weight below 100 kDa, so it is named as Lumbricus Low Molecular Weight Proteins (LLP).[2] This enzyme can be transported to the bloodstream via intestinal epithel.[7] As a drug that consists of serine protease enzyme, it is suspected that the mechanism of action of lumbrokinase, especially as fibrinolytic and antithrombotic. In vitro study by Trisina et al[2] showed that DLBS1033 has fibrinogenolytic activities on fibrinogen α, β, and γ chain, decreasing platelet aggregation and prolong clotting time.[2]

Plasma half-life is a very important parameter to calculate the dose and interval administration of DLBS1033. The active fraction of DLBS1033 in plasma could not be measured since the active isoenzyme is still unknown. Therefore, the aim of this study was to evaluate the biological half-life of DLBS1033 by measuring serial plasmin-antiplasmin (PAP) complex. PAP complex is a stable and inactive compound as a result of fibrinolysis process which is started with activation of plasminogen to plasmin. This activation will produce free plasmin that binds to antiplasmin and becomes PAP complex.[8-10]

METHODS

This study was an open label clinical trial, which was done at Pharmacology and Therapeutic Department and Clinical Pathologic Department of Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo Hospital in July - December 2012. The study drug was enteric coated DLBS1033 tablet, which contained 490 mg bioactive protein fraction; and produced by PT Dexa Medica, Tangerang, Indonesia.

Subjects

This trial was conducted on 14 healthy male subjects. Subjects were considered healthy if vital signs, physical examination, and hematological parameters was within normal range. Other inclusion criteria were male, 18-55 years old, body mass index of 18-25 kg/m^2, gave written informed consent and have PAP complex level between 0-514 ng/mL. Exclusion criteria were subjects with cardiovascular disease, diabetes mellitus, and dyslipidemia, creatinine serum (normal 0,5-1,5 mg/dL) of more than 1.5 x ULN (upper limit normal), SGOT (normal 10-35 Unit) and SGPT (normal 10-40 Unit) of more than 3 x ULN, blood pressure ≥ 140/90 mmHg, fasting blood glucose >126 mg/dL, alcoholic patients, those who took any medications (including traditional medicines, supplements, and vitamins) one week before the study, had bleeding history with unclear etiology, hemoglobin level <10 g/dL, thrombocyte count <100.000/µL and a heavy smoker (Brinkman Index >600). Brinkman index was calculated as the product of the number of tobacco smoke per year. Brinkman index was calculated as tobacco smoke per day times number of year of active consumption. Based on Brinkman index, smokers was divided into two groups, heavy smoker (Brinkman index ≥ 600) and light smoker (Brinkman index <600).[11]

Subjects was dropped out if there were hypersensitivity reactions because of study medicine, subject were not taking the studied medicine as instructed, or taking any other drugs when they were in this trial without permission from investigator. Subjects were divided into two groups, one group for single dose administration and one group for steady state condition.

Ethical Approval and Informed Consent

The Committee of Medical Research Ethics of Faculty of Medicine, University of Indonesia has reviewed the study protocol and issued the approval letter on 25th July 2011. Consent and signed informed consent to enter the study was obtained from each participant after a full explanation and information leaflet has been given. This study was also registered at clinicaltrials.gov NCT01905878.

Study Procedure

Potential subjects were invited to receive explanation of the study. After informed consent form had been signed, investigator collected the subject's medical history, data on alcohol consumption habit, data on medication that had been taken seven days before study (including traditional medicine and supplement), smoking habit, bleeding history with unknown etiology, and calculated the subject's body mass index based on body weight and height measurement. Subjects also undergone a clinical assessment including vital signs, and laboratory assessment of routine hematology, fasting blood glucose, liver function test (SGOT and SGPT), renal function test (serum creatinine), thrombocyte aggregation and PAP complex.

Procedure

Eligible subjects were randomized to receive single dose (3 x 490 mg) or repeated dose until steady state conditions (3 x 490 mg/day for 3 days).

The eligible subjects of single dose group were instructed to come to study site on the scheduled day. Blood samples were collected to assess PAP complex concentration , serum creatinine, SGOT, SGPT, PT (prothrombin time) and aPTT (activated partial thromboplastin time). Subjects were instructed to take three tablets of enteric coated DLBS1033 in front of investigator with 240 mL of water. Blood samples were collected on 30 minute, 60 minute, 90 minute, 2 hour, 3 hour, 4 hour, 6 hour, 8 hour, 10 hour, 12 hour, and 24 hour to evaluate serial PAP complex.

The eligible subjects of steady state condition group instructed to come to study site on the scheduled day (day 1 and day 4). On day 1

blood samples collected to assess PAP complex concentration, serum creatinine, SGOT, SGPT, PT, and aPTT. Subjects instructed to take one tablet enteric coated of DLBS1033 with 240 mL of water in front of the investigator. Study drug package (consist of 8 tablets) dispensed to the subjects at day-1, and they were instructed to take the drug three times daily 30 minutes before meals. Subjects also were instructed to record any adverse events and concomitant medication prescribed in diary card. Subjects had to take the drug for three days (day 1, 2, and 3). On day 4, subjects were instructed to come to study site. Blood samples were collected at time 0, 30 minute, 60 minute, 90 minute, 2 hour, 3 hour, 4 hour, 6 hour, 8 hour, 10 hour, 12 hour, and 24 hour to evaluate serial PAP complex.

All subjects were asked to stay for two nights in PT. Equilab International Laboratory staying room. Subjects were only permitted to eat food from investigator and not permitted to smoke. Subjects were requested to fast for 10 hours before drug administration for single dose group and prior plasma sampling for steady state group. All subjects were undergone a clinical assessment including vital signs and the presence of adverse events at the time of blood sampling. Any adverse events were recorded on case report form (CRF).

Statistical Analysis

Demographic, clinical, and laboratory data were recorded in case report form for each subject. Adverse events were evaluated by investigator and recorded in adverse event form in case report form. Serial PAP complex concentrations were analyzed as descriptive data.

RESULTS

The result of demographic and laboratory screening were shown on **Table 1** and **Table 2**.

Table 1. Demographic characteristics of study subjects

Variables	Mean (SD)	
	Single dose group (n=7)	Steady state group (n=7)
Age (years)	23.8 (4.78)	32.4 (10.69)
Height (cm)	166 (6.11)	161 (5.07)
Weight (kg)	55.1 (6.01)	55.4 (4.72)
Body mass index (kg/m^2)	20 (2.15)	21.5 (2.31)
Brinkman index	28.3 (25.15)	34.7 (47.68)

Table 2. Laboratory parameters of study subjects at baseline

Variables	Mean (SD)	
	Single dose group (n=7)	Steady state group (n=7)
Hemoglobin (g/dL)	15.5 (1.14)	14.9 (0.77)
Hematocrit (%)	45.3 (3.03)	44 (2.30)
Thrombocyte (/μL)	266 714.3 (50 135.53)	273 714.3 (56 870.36)
SGOT (U/L)	22.9 (7.60)	23.4 (3.91)
SGPT (U/L)	17.3 (14.81)	21.4 (11.84)
Serum creatinine (mg/dL)	0.9 (0.07)	0.9 (0.11)
Fasting blood glucose (mg/dL)	74.9 (8.43)	74.3 (9.43)
PAP complex (ng/mL)	54.2 (9.94)	70.7 (20.78)
Thrombocyte aggregation (%)	78 (8.89)	71.9 (10.67)

Table 3. PAP complex (ng/mL) after single dose DLBS1033 administration (N = 7)

Time of blood sampling (hours)	1	2	3	4	5	6	7	Mean
0	60.59	63.24	58.24	51.47	69.56	44.71	56.32	57.73
0.5	42.21	52.35	58.38	39.26	56.62	45.88	59.12	50.55
2	35.44	42.5	58.97	43.24	53.24	31.76	53.09	45.46
3	41.47	56.18	31.91	27.79	44.85	25.44	42.06	38.53
4	26.62	32.79	31.91	30.44	32.94	34.12	88.09	39.56
5	30	47.06	47.21	31.76	24.12	12.5	36.18	32.69
6	16.62	28.68	37.94	30.15	26.03	10.88	13.97	23.47
8	9.85	33.09	18.24	16.91	21.32	28.68	20.44	21.22
10	15.59	16.76	13.68	16.91	13.82	17.94	21.03	16.53
12	8.53	8.24	12.21	17.21	24.12	9.26	9.12	12.67
14	25.29	11.47	22.94	15.29	27.35	9.56	4.71	16.66
24	2.65	15.74	15	7.94	11.91	10.88	15.88	11.43

Table 4. PAP complex (ng/mL) concentration on steady state condition of DLBS1033 (N = 7)

Time of blood sampling (hours)	8	9	10	11	12	13	14	Mean
0	46.47	55.44	57.79	76.62	46.62	54.85	72.06	58.55
0,5	49.41	55.59	52.21	60.74	56.32	43.53	53.58	53.07
2	26.91	55	42.35	54.26	57.65	47.94	130.15	59.18
3	21.18	39.41	30	42.79	31.32	41.91	70.74	39.62
4	42.5	63.53	36.47	46.03	39.41	22.79	61.47	44.60
5	33.68	34.41	45.59	41.62	19.26	23.68	54.41	36.09
6	29.56	23.82	30.29	46.03	37.5	34.85	55.44	36.78
8	17.65	18.97	26.03	17.35	17.65	30.29	46.32	24.89
10	9.71	13.38	17.21	17.35	10.59	23.97	28.38	17.23
12	16.62	11.18	17.21	28.68	23.97	21.91	32.06	21.66
14	10.74	14.85	12.65	23.68	20.74	8.38	26.76	16.83
24	9.71	10.44	8.38	14.41	19.12	30.74	87.5	25.76

Biological half-life of DLBS1033 in Single Dose Administration

Serial PAP complex parameter after single dose administration of DLBS1033 were shown on **Table 3**.

The highest mean of serial PAP complex (y) was 57.73 ng/mL, and was reached prior to drug administration. This result meant that the activity of DLBS1033 after single dose administration was too small to determine.

Biological Half-life of DLBS1033 in Steady State Condition

From data in vivo trial, the half life of DLBS1033 is 8 hours. It was predicted that 3 x 490 mg DLBS1033 per day for three days administration was enough to reach steady state condition. The result of serial PAP complex concentration from steady state group is shown on **Table 4**.

The biological half-life of DLBS1033 was calculated based on mean of PAP complex on each time sampling from seven subjects. Figure 1 showed the mean PAP complex on steady state condition of DLBS1033.

In steady state condition, the PAP complex concentration increased in 2 hours after last drug administration. From **Figure 1**, the linier equation of mean serial PAP complex was y = - 2,9087x + 54,556. The highest mean of serial PAP complex (y) was 59,18 ng/mL. That value become 29,59 ng/mL in:

y = - 2.9087x + 54.556

29.59 ng/mL = - 2.9087x + 54.556

x = 8.6 hours

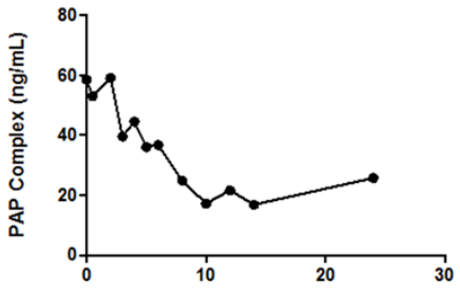

Figure 1. Mean of serial PAP complex in each time sampling of 7 subjects from steady state condition of DLBS1033 group

In steady state condition, the biological half life of DLBS1033 was 8.6 hours.

Safety Parameters

There were five laboratory parameters that measured as safety parameters i.e. PT, aPTT, SGOT, SGPT, and creatinine serum. There were no significant findings on all laboratory parameters, only four adverse events from 14 subjects (**Table 5**) and no serious adverse events in this study.

Table 5. Adverse events of single dose and steady state subject group (N = 14)

No.	Adverse events	No of subjects Single dose group	No of subjects Steady state group
1	Headache	1	-
2	Dizziness	1	-
3	Myalgia	1	1
	Total	3	1

DISCUSSION

DLBS1033 is a lumbrokinase extract obtained from Lumbricus rubellus that possesses eight isoenzymes. Similar to other lumbrokinase, we predict that the mechanism of action of DLBS1033, especially as plasminogen activator. This prediction is supported by the result in an in vitro study by Trisina et al[2], which showed that DLBS1033 has fibrinogenolytic activities, decreasing platelet aggregation and prolong clotting time. As a plasminogen activator, DLBS1033 will activate plasminogen to be plasmin. The free-plasmin will bind to antiplasmin and form plasmin-antiplasmin complex (PAP complex). The PAP complex concentration was used as a fibrinolytic parameter on this study.[9,10]

Coagulation and fibrinolysis are a physiological process which is controlled by several factors. These two processess control our blood fluidity. Fibrinolysis process starts when fibrins are deposited as a product of coagulation process. Fibrin regulates its own degradation by bind to plasminogen and tPA. This binding activate tPA to convert plasminogen to plasmin. Once formed, plasmin cleaves fibrin.[9,13] Plasmin binding-fibrin will degrade fibrin clot to be fibrin degradation product, and degrade cross-linked fibrin to be D-dimer.[8,9] Free plasmin will bind to antiplasmin and become plasmin-antiplasmin complex (PAP complex) which is stable and inactive.[10] Normal concentrations of PAP complex are different among individuals. It depends on some factors, such as inflammation; however, high level of PAP complex concentration is not directly linked to inflammation.[14]

On single dose of DLBS1033 (**Table 3**), the PAP complex was not increased after drug administration. The highest PAP complex concentration was reached before drug administration. This result indicated that the activity of DLBS1033 was too small to detect after single dose administration. DLBS1033 is an oral drug, and it needs to be absorbed and might pass the first metabolism process before exerting its effect.[7] It might need few hours to reach steady-state condition to measure the DLBS1033 fibrinolytic activity. The biological half-life of DLBS1033 on steady state group was 8.6 hours. This result indicated that the effect as fibrinolytic could be measured after steady state condition was reached. The increase of PAP complex concentration level in 2 hours also showed that the absorption was relatively fast and it was ensured that the absorbed DLBS1033 components were active form.[12]

The drug profile in steady state condition is important information for long term used drug. As an oral fibrinolytic drug, DLBS1033 can be used for long period in some clinical conditions, such as post cardiovascular events. One pilot study was conducted to evaluate the efficacy of oral lumbrokinase for stable angina pectoris. On that trial, ten subjects were given two tablets of 250 mg oral lumbrokinase, three times daily, for 30 consecutive days. It is concluded that the drug could improve the myocardial perfusion in patients.[15] This result needs to be extrapolated to the bigger trial with more subjects. In current clinical practice, DLBS1033 usually use as an adjuvant drug for standard treatment.

In our study, quantification of half-life used pharmacodynamics parameters, since the real elimination half-life of this drug is still unknown. The DLBS1033 half-life quantification could be more accurate if the concentration of DLBS1033 protein fraction in plasma can be quantified.

CONCLUSION

On this study, we concluded that the fibrinolytic effects of DLBS1033 might be measured in steady state condition. The biological half-life of DLBS1033 in steady state condition was 8.6 hours. There were no serious adverse events on the two groups. Result of this study can be used as a reference to quantify dose regimen for the next clinical trial using this drug.

CONFLICT OF INTEREST

This trial was funded by Dexa Laboratories of Biomolecular Sciences, Dexa Medica, Cikarang, Indonesia.

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