

## Improved Myocardial Perfusion in Stable Angina Pectoris by Oral Lumbrokinase: A Pilot Study

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### Abstract

**Objective:** A novel antithrombotic agent, lumbrokinase, was evaluated in this pilot study for its efficacy in the treatment of symptomatic stable angina.

**Design:** This was a single-armed cohort study.

**Settings:** The study was conducted at the National Cardiovascular Center, Harapan Kita, Jakarta, Indonesia.

**Subjects:** The study comprised 10 patients who had coronary artery disease and stable angina and who consented to the trial.

**Intervention:** Patients were treated with oral lumbrokinase for 30 consecutive days in addition to their standard medical therapy.

**Outcome measures:** Stress technetium-99m sestamibi myocardial perfusion imaging (MPI) was performed before and at the conclusion of the active treatment period. The degree and extent of inducible ischemia observed on the myocardial perfusion images were evaluated using the previously validated semiquantitative indices (Summed Stress Score and Summed Difference Score).

**Results:** Following active treatment, the mean Summed Stress Score and Summed Difference Score decreased by 39% and 37%, respectively. The anginal symptom was ameliorated in 6 of 10 patients. No adverse reaction including major or minor bleeding was observed.

**Conclusions:** This paper represents the first description of the use of oral lumbrokinase in the treatment of chronic coronary artery disease with objective assessment using MPI. Oral lumbrokinase improves regional myocardial perfusion in patients with stable angina.

### Introduction

STABLE ANGINA AFFECTS AN ESTIMATED 2%–4% of the population in the developed nations, with an annualized mortality of 0.9%–1.4%.<sup>1</sup> Over half of patients with stable angina report being severely limited in their everyday activities,<sup>2</sup> and stable angina is often considered as more an issue of quality of life than mortality.<sup>3</sup>

The condition almost always relates to atheromatous obstruction of coronary arteries, with resultant failure to meet myocardial oxygen demand during periods of exercise or mental/emotional stress culminating in myocardial ischemia and its manifest symptoms.

In patients with chronic coronary artery disease (CAD) and stable angina, three groups of medications—the  $\beta$ -blockers, calcium channel blockers, and nitrates—have been the

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mainstay of the anti-anginal armamentarium,<sup>4</sup> while anti-thrombotic and antiplatelet agents have been largely employed as an adjunct therapy to modify the risk of future cardiac events.<sup>1,5,6</sup>

An earthworm (*Lumbricus rubellus*) in the form of raw animal "herb," has been used as a traditional medicine in China for several thousand years,<sup>7</sup> and extract from the earthworm has been empirically used in Asia for the treatment of vascular disorders. Recent clinical trials confirmed its clinical efficacy in the treatment of CAD and thrombotic cerebral infarct,<sup>8,9</sup> albeit publication related to the former has been largely confined to the Chinese language literature. Lumbrokinase—the term given to the group of enzymes extracted from the earthworm—possesses plasminogen-activating and direct fibrinolytic properties.<sup>7</sup> The chemical definitions and amino acid sequences of each of the six isoenzymes of lumbrokinase with their respective fibrinolytic activities have previously been identified and characterized.<sup>10</sup>

We undertake this pilot study to objectively evaluate the effect of lumbrokinase on regional myocardial perfusion among patients with CAD and symptom of stable angina.

## Materials and Methods

### Study population

Following informed consent, 10 patients from the outpatient clinic of Harapan Kita National Cardiovascular Center were enrolled in an open-label single-arm study. The study protocol was approved by the Ethics and Human Research Committee of the National Cardiac Centre, Jakarta. All patients had CAD confirmed on coronary angiography, symptom of stable angina, and were on stable cardiac medications including low-dose (80-mg) prescription aspirin. None was on warfarin, nor had any of the patients received previous fibrinolytic therapy. Table 1 shows the population demographics.

### Intervention

Two (2) capsules of Tromboles<sup>®</sup> (The Institute of Biophysics, Chinese Academy of Sciences, Beijing), each containing 250 mg lumbrokinase extract equivalent to 300,000 U of lumbrokinase derived from an artificially cultured *Lumbricus* strain, were administered three times daily for 30 consecutive days. Pre-existing medical therapy was maintained throughout the study period. All 10 patients completed the study. Myocardial ischemia was assessed by technetium-99m sestamibi myocardial perfusion imaging (MPI) using single photon emission tomography, performed within 1 week prior to commencement of the trial and repeated during the last week of active treatment.

### Adenosine MPI protocol

Myocardial perfusion was assessed using adenosine pharmacologic stress as previously described.<sup>11</sup> All patients were instructed not to consume caffeine-containing products for 24 hours prior to testing. A resting MPI was performed 1 hour following bolus intravenous injection of 350 MBq Tc-99m sestamibi. Three (3) hours later, adenosine was infused for 6 minutes, at 140  $\mu$ g/kg/min. At the end of the third minute of infusion, Tc-99m sestamibi (900 MBq) was injected. MPI acquisition was started 60 minutes following radiotracer in-

TABLE 1. BASELINE CHARACTERISTICS

<i>Characteristic</i>	
Age	58.9 $\pm$ 7
Weight	66.7 $\pm$ 9.4
Chest Pain history (months)	16 $\pm$ 14
Prior Myocardial Infarction	1 of 10
Diabetes	2 of 10
Hypertension	7 of 10
HDL (mg/dL)	38.8
Triglycerides (mg/dL)	196.9
LDL (mg/dL)	111.8
<i>Medication</i>	
Aspirin	10 of 10
Beta blockers	7 of 10
Calcium antagonists	3 of 10
Nitrates	10 of 10

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

jection. Whenever possible, pharmacologic stress was augmented using low-level treadmill or handgrip exercise.

### MPI acquisition protocol

The MPI acquisitions were performed as previously described, employing a circular 180° orbit for 64 projections at 25 seconds/projection.<sup>12</sup> All images were subject to quality-control measures. No attenuation or scatter correction was used. After filtered backprojection, short-axis, vertical long-axis, and horizontal long-axis tomograms were generated.

### Stress ECG

During adenosine infusion, 12-lead electrocardiogram was recorded each minute, with continuous monitoring of leads aVF, V<sub>1</sub>, and V<sub>5</sub>.

### Image interpretation

Semiquantitative visual interpretation of MPI images used short-axis and vertical long-axis tomograms divided into 17 segments for each patient and each segment was scored by consensus of 2 expert observers using a five-point scoring system (0 = normal, to 4 = absence of segmental uptake).<sup>12</sup> Three (3) global perfusion indexes were employed to combine assessments of perfusion defect extent and severity.<sup>13</sup> By adding the 17 segment scores, summed stress scores (SSS) and summed rest scores (SRS) were calculated.<sup>14</sup> The difference between the SSS and SRS was defined as the summed difference score (SDS), assessing myocardial perfusion defect reversibility. SSS <4 was defined as a normal scan; SSS 4–8, 9–13, and >13 were defined as mildly, moderately, and severely abnormal scans, respectively.<sup>14</sup> The SDS <2 was considered no ischemia, 2–7 mild to moderate, and >7 severe ischemia.<sup>15</sup>

The differences in the extent and degree of inducible ischemia between the initial and following active treatment MPI were represented by delta SSS and delta SDS.<sup>14,15</sup>

### Follow-up data

Follow-up interviews and physical examinations were performed periodically during and within 1 month following completion of the active treatment period.

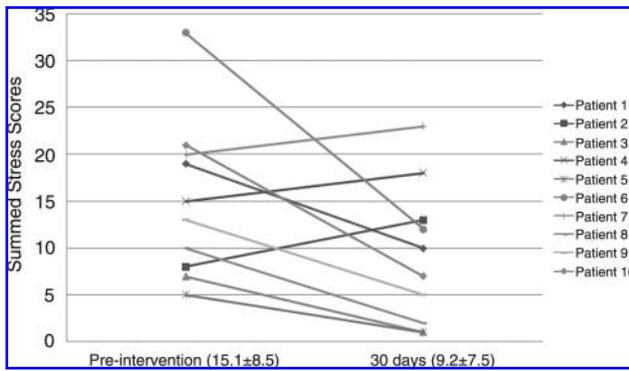


FIG. 1. Change in degree of myocardial perfusion abnormality by myocardial perfusion imaging after 30-day treatment with lumbrokinase.

Statistical analysis

Formal statistical analysis was not included due to the small size of the study population.

Results

Following 1 month of active treatment, the mean SSS was reduced from  $15.1 \pm 8.5$  to  $9.2 \pm 7.5$  (Fig. 1). The  $\delta$  SSS was  $5.9 \pm 8.1$ , reflecting a mean reduction of 39% in SSS. Figure 2A shows the number of patients in each of the four levels (normal to severe, as previously defined) of SSS abnormality, before and after treatment with lumbrokinase.

SDS was also found to have been reduced following active treatment. The mean SDS decreased from  $12 \pm 6.5$  to  $7.6 \pm 7.0$ . Delta SDS was  $4.4 \pm 8.5$ , reflecting a mean reduction of 37% in SDS. Figure 2B shows the number of patients in each of the four levels (normal to severe, as previously defined) of SDS abnormality, before and after treatment with lumbrokinase.

Angina was ameliorated in 6 patients. No adverse reaction including major or minor bleeding was observed.

Table 2 summarizes the changes in MPI indices and symptom response with active treatment. A case example of a patient's pretreatment and post-treatment MPI is shown in Figure 3.

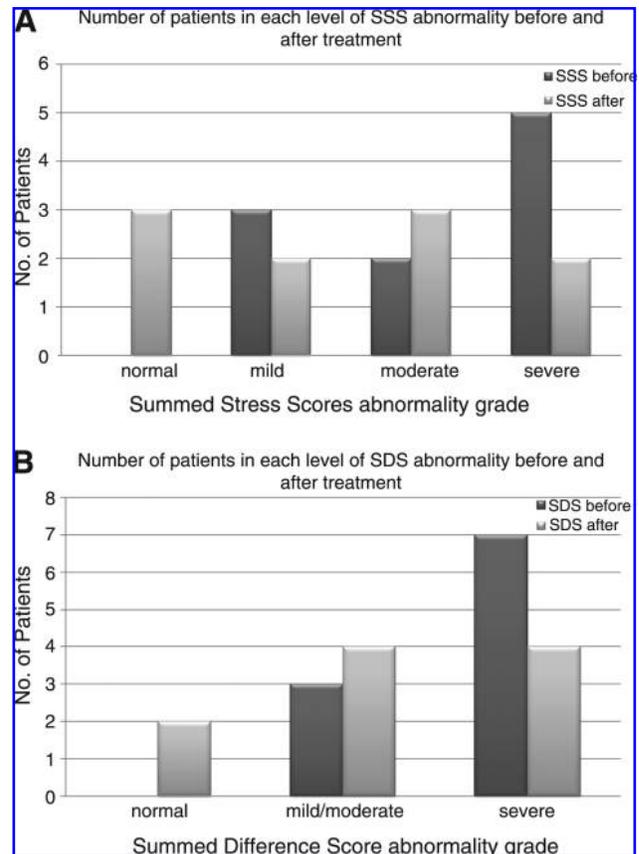


FIG. 2. (A) Number of patients in each level of summed stress scores (SSS) abnormality before and after treatment. (B) Number of patients in each level of summed difference score (SDS) abnormality before and after treatment.

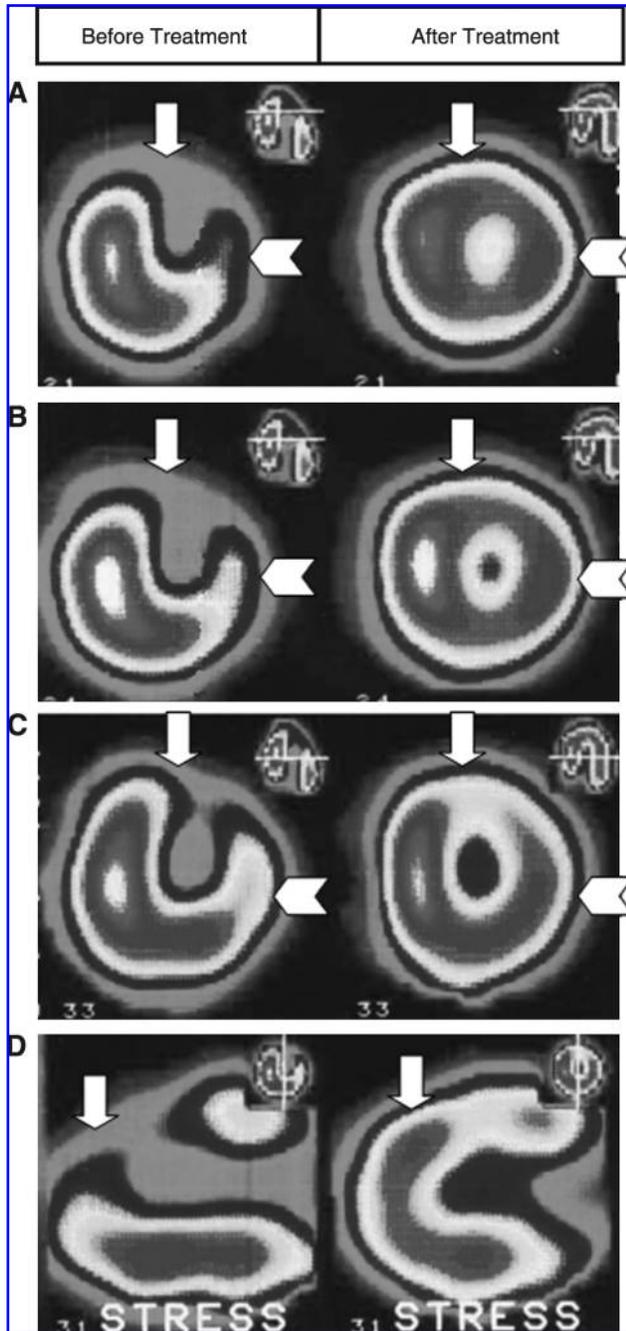
Discussion

The symptom of stable angina is the somatic manifestation of myocardial ischemia, which in turn is due to the composite effects of atherosclerotic burden, platelet and coagulation function, blood viscosity, integrity of endothelial function, and coronary flow reserve. In addition to directly reducing myocardial blood supply, atherosclerosis has been found to

TABLE 2. CHANGES IN MYOCARDIAL PERFUSION IMAGING INDICES AND SYMPTOM RESPONSE WITH ACTIVE TREATMENT

Patient	SSS before trial	SSS after trial	Change in SSS	SDS before trial	SDS after trial	Change in SDS
1	19	10	-9	13	6	-7
2	8	13	5	6	14	8
3	7	1	-6	5	1	-4
4	15	18	3	14	20	6
5	5	1	-4	5	1	-4
6	33	12	-21	27	8	-19
7	20	23	3	13	17	4
8	10	2	-8	9	2	-7
9	13	5	-8	13	5	-8
10	21	7	-14	15	2	-13
Mean	15.1	9.2	-5.9	12	7.6	-4.4

SSS, summed stress scores; SDS, summed difference score.



**FIG. 3.** A case example of a patient's stress myocardial perfusion before and at the end of 30-day treatment with lumbrokinase. Corresponding representative apical (A), mid (B), and basal (C) short axis, and midvertical long axis (D) tomographic perfusion images demonstrating significant reduction in the extent and degree of inducible ischemia in the anterior (arrows) and lateral (arrowheads) left ventricular wall.

promote platelet aggregation,<sup>16,17</sup> while platelets and fibrin-like products are constituents of the intima in atherosclerotic lesions. It has also been acknowledged that fibrinogen levels, known to be elevated in patients with CAD, play an important role in blood viscosity.<sup>18</sup>

Antithrombotics and antiplatelets are primarily used in symptomatic patients with CAD for prognostic modification

rather than management of symptoms related to myocardial ischemia.<sup>1</sup> Beyond this application, several trials have shown the positive effects of these agents in the management of angina among patients with stable CAD.

In the Thrombosis Prevention Trial,<sup>19</sup> Knottenbelt and co-workers showed that given to patients at high risk of having significant coronary atherosclerosis, warfarin reduced the incidence of angina by 16%, and was 37% less among those taking warfarin than in those taking aspirin.

Barron and colleagues<sup>20</sup> studied patients with chronic stable angina treated with 10 days of intravenous heparin or placebo, in addition to conventional therapy. The study showed that compared to placebo, the heparin-treated group achieved 9% and 14% reductions in ischemic extent and severity, respectively, on stress-redistribution thallium-201 tomographic imaging. In another study,<sup>18</sup> Melandri et al. added subcutaneous low molecular heparin (Parnaparin) to standard therapy over 3 months in patients with stable angina. The treatment group demonstrated significant improvement in the time to significant ST depression and reduction of peak ST depression with exercise, as well as reduction in the severity and frequency of anginal symptoms.

In this pilot study, we demonstrated that 30 days' administration of lumbrokinase, a fibrinolytic and plasminogen activating agent, was associated with an objective reduction in myocardial ischemic extent and severity among patients with known CAD and symptom of stable angina. The 6 patients who reported resolution of or a reduction in angina duration and frequency all had objective evidence of reduction in inducible ischemic extent and severity. Conversely, the absence of symptom improvement in the remaining patients was accompanied by the lack of improvement in myocardial perfusion pattern on adenosine sestamibi imaging except for 1 patient who had an improved MPI without amelioration of the anginal symptom.

It is of interest to note that all of the seven patients who demonstrated objective reduction in the degree and extent of inducible ischemia by MPI had pre-existing metabolic syndrome, whereas metabolic syndrome was present in only 1 of the 3 patients who did not show ischemia reduction. Metabolic syndrome has been found to be associated with inhibited fibrinolytic function and increased prevalence of atherosclerosis,<sup>21</sup> which may relate to the observation in our study.

The exact mechanism by which lumbrokinase administration affected the observed results is uncertain. Lumbrokinase may exert anti-ischemic effects by pathways known to be associated with fibrinolytic agents such as modification in coagulation function, direct effects on endothelial function<sup>22</sup> or promoting angiogenesis.<sup>23</sup>

#### Safety profile

No adverse reaction or events including bleeding were observed in this open-labeled pilot study. Although lumbrokinase is an SFDA (State Food and Drug Administration, P.R. China)-approved pharmacotherapeutic and the Chinese literature is replete with its clinical safety data, including its safety profile in terms of hemorrhagic diathesis,<sup>24,25</sup> the safety profile of lumbrokinase in patients with CAD (who are likely to receive a variety of and often multiple antiplatelets and/or anticoagulants) has yet to be formally evaluated.

Future research should expand on the published results of this pilot study and explore the therapeutic dose range in both experimental and clinical studies. In addition, the safety profile in relevant patient cohorts should be systematically assessed in prospectively conducted clinical trials.

#### Potential clinical applications

Currently, there is no oral form of fibrinolytic in the Occidental pharmacotherapeutic armamentarium. The parenteral fibrinolytics are relatively short acting and are used only in patients with acute myocardial or thrombotic cerebral infarctions. Thus, lumbrokinase, being a unique oral fibrinolytic with some data reporting its efficacy in experimental acute myocardial infarction<sup>26</sup> and clinical thrombotic stroke,<sup>8</sup> may have an adjunct or complementary therapeutic role in patients presenting with acute cerebro- or cardiovascular events.

In a chronic setting, the addition of lumbrokinase with primarily fibrinolytic properties augments the number of available oral antithrombotic agents that are currently limited to the realm of antiplatelets (aspirin, dipyridamole, and adenosine diphosphate inhibitors such as clopidogrel) or anticoagulants (e.g., warfarin). In our study, all the patients were already on aspirin and anti-anginal therapy. The addition of lumbrokinase not only objectively reduced the degree and extent of inducible ischemia but importantly also provided anginal symptom relief not obtained previously, thus fulfilling one of the primary goals of therapy for patients with stable angina (i.e., relief of symptoms with resultant improvement in quality of life).<sup>3</sup>

In addition, since inflammation is a key aspect of atherosclerotic progression and plaque instability,<sup>27</sup> the possible anti-inflammatory properties of lumbrokinase through its recently reported antiplatelet effects<sup>28</sup> may impose yet another dimension in its clinical application. Further robust research should be undertaken to determine the therapeutic and prognostic scope of lumbrokinase in chronic and acute cardiovascular disorders.

#### Conclusions

This pilot study represents the first description of the use of oral lumbrokinase in the treatment of chronic CAD with objective assessment using MPI. Lumbrokinase effects a reduction in the degree and extent of inducible myocardial ischemia with amelioration of anginal symptoms in patients with CAD with stable angina. Randomized, placebo-controlled trials involving relevant patient cohorts are deemed warranted to further evaluate the scope of clinical utility of this novel oral anti-anginal agent.

#### Acknowledgments

This paper was presented in part at the 16th ASEAN Congress of Cardiology, Bali, April 2007. The study was funded through an internal grant, Department of Cardiology and Vascular Medicine, Harapan Kita National Cardiovascular Center, Jakarta, Indonesia. Lumbrokinase (Tromboles<sup>®</sup>) was supplied by Bio-life Products, Jakarta, Indonesia.

#### Disclosure Statement

No competing financial interests exist.

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