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Phase I Clinical Trial to Evaluate the Safety and Tolerability of Oral SN-1 Extract Formulation in Healthy Thai Volunteers

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Abstract

The human immunodeficiency virus (HIV) is one of the greatest current worldwide health threats. With the difficulties faced from the adverse effects of drugs used to treat HIV and the emergence of drug resistance, a new drug or medicinal herb is urgently needed. Previous study by the Department of Medical Science, Ministry of Public Health, Thailand, has demonstrated that the SN-1 extracts of the Thai medicinal herb "Khailung" (*Phyllanthus* sp) is a potent inhibitor of the enzyme HIV-1 reverse transcriptase *in vitro*. We conducted a study comparing an oral formulation of a 500 mg/day dose of SN-1 extract given for 14 days, and an oral formulation of SN-1 extract 1,000 mg/day for 14 days, to determine safety and tolerability. The study population consisted of 16 healthy Thai volunteers residing in Bangkok. Routine laboratory and physical examinations were performed every day during drug administration, and weekly during follow-up for 28 days after final drug administration, to observe any adverse effects. The results showed no finding of clinically significant abnormalities or changes from the baseline for routine laboratory, vital signs and body weights compared on any follow-up day. In addition, no clinical adverse reactions or drug-related effects were observed during the study. We conclude that the oral formulation of SN-1 extract was well-tolerated. The clinical pharmacology of the SN-1 extract should be further studied to explore the potential for using this drug to treat HIV-1.

Keywords: HIV, SN-1, Khailung, *Phyllanthus* sp, HIV-1 reverse transcriptase

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Introduction

Human immunodeficiency virus (HIV), the etiologic agent of acquired immune deficiency syndrome (AIDS), is estimated to have infected over 60 million people. Infection with HIV type I (HIV-I) is prevalent throughout the world, and is characterized by a progressive deterioration of the immune system, which is almost uniformly fatal if

untreated. This pandemic is now the fourth leading cause of mortality worldwide, with over 95% of deaths occurring among young adults living in the developing world. HIV remains a major public health problem in epidemic regions [1].

The current anti-HIV drugs approved by the US Food and Drug Administration (FDA) are targeted to HIV-1 reverse transcriptase and proteases [2]. More and more patients with HIV infection and/or AIDS are unable to use these drugs because of the serious adverse effects and the development of viral drug resistance. This creates a need for new drugs that are well-tolerated and simple to use [3-4]. At present, a variety of natural products have been found to inhibit enzymes crucial to the life cycle of HIV, including HIV-reverse transcriptase, HIV-protease and HIV-integrase [5]. In Thailand, the Division of Medical Science, Ministry of Public Health has studied the extracts of Thai medicinal herbs reputed in Thai folklore to have anti-infection properties [6-7].

SN-1 extract, one of the Thai medicinal herb extractions, exhibits a significant activity against enzyme HIV-1 reverse transcriptase. SN-1 is an alcohol extract of the Thai medicinal plant, "Khailung" (*Phyllanthus* sp), in the Family Euphorbiaceae. Khailung has been used as a folk remedy to treat warts. However, the structure and molecular weight have not yet been clearly identified, but its anti-enzyme HIV-1 reverse transcriptase activity has been studied *in vitro*. The results showed that SN-1 extract was potentially a rich source for inhibiting enzyme HIV-1 reverse transcriptase [8-9]. In addition, acute and chronic toxicity testing have been conducted in rats. No adverse reactions were observed [10]. The objective of this study was to describe the safety and tolerability of two oral doses, 500 mg/day *vs* 1,000 mg/day, of SN-1 extract in healthy Thai volunteers using a randomized non-placebo controlled, two-arm phase I clinical trial.

Materials and methods

Study volunteers

Healthy male and female volunteers, aged 20-50 years, weighing 45.0 to 75.0 kg, and living in Bangkok were eligible for the trial. Inclusion criteria included: non-lactating and non-pregnant (in females), no significant abnormal findings on history or examination—particularly liver, kidney, cardiovascular disease or peripheral neuropathy—plus no history of receiving another experimental drug within the past 30 days. Volunteers had to be able to remain hospitalized in the Bangkok Hospital for Tropical Diseases for 14 days during drug administration, or more as medically necessary, and come for follow-up weekly to complete a 42-day follow-up to observe any adverse effects. Female and male participants were required to use suitable barrier contraception during the study. The trial discontinued volunteers with allergy or sensitivity to the study drug or inability to tolerate the study drug. Written informed consent for participation was obtained from all volunteers before starting the study. The Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, approved the study.

At enrolment, a medical history was taken, including a full physical examination, chest x-ray, and routine laboratory investigations (biochemistry, complete blood count, urinalysis (including urine pregnancy test in females), and anti-HIV antibody).

Drug administration and study design

The phase I clinical trial of the oral SN-1 extract formulation was conducted at the Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University. The trial was a randomized non-placebo controlled, two-arm phase I clinical trial between two different doses of oral SN-1 extract formulation, *ie* 500 mg/day *vs* 1,000 mg/day for 14 days. The oral SN-1 extract formulation was developed by Medicinal Plant Research Institute, Department of Medical Sciences, Ministry of Public Health, Thailand. Volunteers were randomly assigned to one of the two groups. Subjects in the first group received one 250 mg capsule of the oral SN-1 extract formulation twice a day for 14 days. The second group of eight subjects received two 250 mg capsules of the oral SN-1 extract formulation twice a day for 14 days. The study drugs were administered after meals. Each dose and timing of administration was documented. Any dose that was vomited within one hour of administration

was repeated once on that day only. Investigators were informed of any repeated doses. The study nurse observed each subject for two hours after study drug administration, recording vomiting or other outcomes.

Compliance with all drug intakes was under the investigators' supervision. No other concurrent drug or alcohol was taken for the two weeks prior to, or during, the study period.

Blood and urine sampling

Seven ml blood samples and 10 ml urine samples were collected at screening [Day (-7)] and on Days 1, 3, 7, 10, 14 and 42 of treatment. The following data were collected:

- Serum chemistry profile: electrolytes, blood urea nitrogen, creatinine, total protein, albumin, aspartate and alamine aminotransferases, alkaline phosphatase, amylase, total and direct bilirubin, and fasting blood sugar.
- Hematology profile: red blood cell count, hemoglobin, white blood cell count with differential, and platelet count.
- Urine analysis: specific gravity, pH, occult blood, glucose, ketone, and protein.
- Chest x-ray, serum anti-HIV, and urine pregnancy tests (in females) were performed on the screening day only.

Monitoring for safety

The volunteers were physically examined and adverse reactions during the study were recorded with the date and time at which they occurred and disappeared. Adverse effects were assessed on the basis of non-suggestive questioning by the study investigators. These included gastrointestinal, central nervous, cardiovascular, and dermatological effects, and other changes possibly attributable to the study drug. Routine blood investigations (hematology and biochemistry), and urinalysis were performed prior to, and at the end of the study (seven days before starting drug administration and at the follow-up visit until 28 days after the last drug administration).

Statistical analysis

Statistical analysis was performed using Analyze It Add Ins for Excel for Windows [11]. All the *P-values* reported are from 2-tailed tests and the statistical significance level was set at 0.05. The distribution of data was assessed for normality using the Shapiro-Wilk test. Data were expressed as means and SD. Three statistical tests were performed: 1) chi-square analyses to test differences between two groups of the qualitative variables, 2) independent t-test to test differences between two groups of quantitative variables in demographics and baseline laboratory data, and 3) repeated measure ANOVA to test differences in each item of the laboratory data at each follow-up, with respect to data baseline levels [12].

Results

A total of 16 volunteers (8 in each group) participated in this trial. All pregnancy tests in female volunteers were negative. All volunteers completed the study as planned. At enrolment, volunteers in both treatment groups were healthy, as verified by laboratory results, vital sign monitoring, physical exam and chest x-ray. Volunteers showed no abnormal signs or symptoms and all of the baseline laboratory data and baseline vital signs were within normal ranges [13]. Table 1 shows the demographic and baseline characteristics of the study volunteers. Five male and 11 female volunteers, aged 24-50 years, participated in the study. There were no significant differences in the distribution of demographic, clinical and laboratory data between the two treatment groups.

All volunteers in this study showed a prompt response to both study drug regimens. Table 2 shows laboratory data for the 16 healthy volunteers during the study. Using repeated measure ANOVA we compared the differences between all laboratory tests during follow-up. The results showed no significantly different changes in laboratory data in hematology and biochemistry tests (both within-group, and between-group effects). There were no findings of clinically significant abnormalities or changes from the baseline for vital signs and body weights among the volunteers (data not shown).

Regarding safety, it was difficult to distinguish between abnormal symptoms and study drug-related side-effects. Some volunteers complained

of abnormal symptoms during drug administration, as follows; headache (2 cases), hunger (1), anorexia (3), diarrhea (1), constipation (2), insomnia (3), and drowsiness (3). However, no serious adverse events were reported during the study.

The overall safety and tolerability of the trial, as assessed by the investigators at day 42, were judged to be "very good" for almost all volunteers in both treatment groups.

Discussion

The phase I trial described in this report has characterized the safety and tolerability of an oral SN-1 extract formulation following oral administration of both 500 mg/day and 1,000 mg/

Table 1 Demographic and baseline laboratory data at screening day {Day (-7)} of 16 healthy Thai volunteers; data are presented as mean and SD.

	SN-1 = 500 mg/day (N = 8)	SN-1 = 1,000 mg/day (N = 8)	P-value
Gender			
Male / Female	2/6	3/5	0.590
Age (years)			
Mean (SD)	37.0 (5.9)	35.8 (7.5)	0.745
Range	28 - 46	24 - 50	
Body weight (kg)			
Mean (SD)	57.0 (6.1)	60.8 (9.2)	0.347
Range	47 - 65	47- 69	
Hematology (Mean (SD))			
Hemoglobin (g/dl)	132.7 (16.3)	136.1 (19.6)	0.715
White blood cells (x 10^9/l)	6.8 (0.5)	7.7 (2.5)	0.332
Platelets (x 10^9/l)	268.7 (44.1)	252.7 (62.5)	0.564
Biochemistry (Mean (SD))			
Total bilirubin (mg/dl)	0.7 (0.3)	0.7 (0.3)	0.953
Direct bilirubin (mg/dl)	0.2 (0.1)	0.2 (0.1)	0.804
Alkaline phosphatase (units/L)	72.3 (13.9)	63.8 (13.8)	0.241
AST/SGOT (units/L)	19.7 (3.5)	21.2 (7.1)	0.605
ALT/SGPT (units/L)	22.7 (9.2)	18.7 (9.1)	0.400
Total protein (g/dl)	7.7 (0.3)	7.6 (0.3)	0.546
Albumin (g/dl)	4.8 (0.2)	4.7 (0.3)	0.539
Globulin (g/dl)	2.8 (0.3)	2.8 (0.3)	0.761
BUN (mg/dl)	9.7 (1.3)	10.6 (2.9)	0.435
Creatinine (mg/dl)	0.7 (0.1)	0.7 (0.2)	0.881
Amylase (unit/L)	68.2 (23.3)	66.1 (17.7)	0.841
Glucose (mg/dl)	95.5 (8.5)	95.5 (15.7)	0.995
Sodium (mmol/l)	140.7 (3.0)	138.6 (2.5)	0.151
Potassium (mmol/l)	4.1 (0.2)	4.1 (0.5)	0.952
Chloride (mmol/l)	104.1 (2.9)	100.8 (3.1)	0.520
Bicarbonate (mmol/l)	24.2 (2.1)	25.0 (2.3)	0.518

Table 2 Laboratory data of 16 healthy volunteers during study {SN-1: 500 mg/day (N = 8); SN-1: 1,000 mg/day (N = 8)}.

Laboratory Data	Mean (SD)						*P-value*	
	Day 1	Day 3	Day 7	Day 10	Day 14	Day 42	Within group	Between groups
Hemoglobin (g/dl)								
SN-1: 500 mg/day	133.3 (17.5)	133.7 (20.0)	133.5 (16.4)	132.3 (17.2)	131.5 (18.5)	131.8 (19.1)	0.695	0.632
SN-1: 1,000 mg/day	138.5 (19.3)	137.5 (18.9)	136.5 (19.2)	137.0 (17.0)	137.6 (18.7)	136.0 (19.2)		
Total	135.9 (18.0)	135.6 (18.9)	135.0 (17.3)	134.6 (16.7)	134.5 (18.2)	133.9 (18.6)		
White blood cells (x 10^9/l)								
SN-1: 500 mg/day	6.9 (1.5)	7.2 (1.4)	7.3 (1.3)	7.2 (1.7)	6.6 (0.8)	7.0 (0.9)	0.830	0.826
SN-1: 1,000 mg/day	8.3 (4.5)	6.8 (2.0)	7.1 (1.9)	7.1 (2.1)	7.3 (2.4)	7.0 (2.1)		
Total	7.6 (3.3)	7.0 (1.7)	7.2 (1.6)	7.2 (1.8)	6.9 (1.8)	7.0 (1.6)		
Platelets (x 10^9/l)								
SN-1: 500 mg/day	256.7 (33.4)	266.1 (32.2)	274.7 (43.2)	270.5 (38.8)	274.2 (37.8)	261.7 (38.8)	0.615	0.510
SN-1: 1,000 mg/day	255.3 (51.6)	253.2 (64.6)	251.0 (54.0)	248.7 (56.1)	251.6 (57.8)	250.6 (54.1)		
Total	256.0 (42.0)	259.6 (49.7)	262.8 (48.8)	259.6 (47.9)	262.9 (48.6)	256.1 (45.8)		
Total bilirubin (mg/dl)								
SN-1: 500 mg/day	0.68 (0.19)	0.66 (0.22)	0.59 (0.04)	0.66 (0.14)	0.59 (0.20)	0.63 (0.13)	0.770	0.739
SN-1: 1,000 mg/day	0.63 (0.16)	0.65 (0.28)	0.62 (0.09)	0.65 (0.19)	0.71 (0.16)	0.71 (0.37)		
Total	0.65 (0.17)	0.65 (0.24)	0.61 (0.07)	0.66 (0.16)	0.65 (0.19)	0.67 (0.27)		
Direct bilirubin (mg/dl)								
SN-1: 500 mg/day	0.30 (0.06)	0.29 (0.04)	0.29 (0.07)	0.31 (0.07)	0.28 (0.09)	0.30 (0.06)	0.583	0.776
SN-1: 1,000 mg/day	0.28 (0.10)	0.26 (0.02)	0.31 (0.08)	0.32 (0.09)	0.33 (0.09)	0.33 (0.30)		
Total	0.29 (0.08)	0.28 (0.03)	0.30 (0.07)	0.32 (0.08)	0.30 (0.09)	0.31 (0.21)		
Glucose (mg/dl)								
SN-1: 500 mg/day	90.2 (6.1)	87.8 (4.6)	90.2 (5.7)	88.0 (5.1)	88.3 (5.2)	89.0 (7.0)	0.798	0.160
SN-1: 1,000 mg/day	85.0 (8.1)	86.3 (6.5)	83.5 (11.9)	86.3 (3.4)	84.0 (8.8)	82.0 (8.7)		
Total	87.6 (7.5)	87.1 (5.5)	86.8 (9.6)	87.1 (4.3)	86.1 (7.4)	85.5 (8.4)		
Alkaline phosphatase (units/L)								
SN-1: 500 mg/day	69.5 (17.3)	71.5 (15.8)	72.5 (16.2)	69.6 (15.3)	71.0 (17.1)	68.5 (13.6)	0.144	0.480
SN-1: 1,000 mg/day	64.0 (14.6)	65.2 (16.4)	68.1 (19.5)	66.7 (21.8)	63.7 (17.4)	59.6 (13.2)		
Total	66.7 (15.8)	68.3 (15.9)	70.3 (17.5)	68.1 (18.3)	67.3 (17.1)	64.0 (13.8)		
AST/SGOT (units/L)								
SN-1: 500 mg/day	27.5 (8.3)	24.3 (3.0)	24.7 (7.1)	27.6 (7.8)	24.0 (7.9)	24.3 (7.2)	0.144	0.445
SN-1: 1,000 mg/day	25.1 (5.9)	23.1 (2.2)	23.8 (5.2)	24.3 (5.4)	23.2 (6.2)	21.1 (5.2)		
Total	26.3 (7.0)	23.7 (2.6)	24.3 (6.0)	26.0 (6.7)	23.6 (6.8)	22.7 (6.3)		
ALT/SGPT (units/L)								
SN-1: 500 mg/day	30.6 (11.1)	28.5 (8.3)	31.6 (15.3)	34.7 (18.4)	30.5 (18.8)	28.3 (14.1)	0.151	0.096
SN-1: 1,000 mg/day	21.3 (8.8)	22.8 (5.8)	21.0 (7.9)	21.6 (8.8)	19.6 (8.1)	17.5 (6.2)		
Total	26.0 (10.8)	25.6 (7.5)	26.3 (13.0)	28.1 (15.5)	25.0 (15.1)	22.9 (11.9)		

Table 2 Laboratory data of 16 healthy volunteers during study {SN-1: 500 mg/day (N = 8); SN-1: 1,000 mg/day (N = 8)} (Continued).

Laboratory Data	Mean (SD)						*P-value*	
	Day 1	Day 3	Day 7	Day 10	Day 14	Day 42	Within group	Between groups
Total protein (g/dl)								
SN-1: 500 mg/day	7.4 (0.4)	7.4 (0.3)	7.4 (0.3)	7.4 (0.1)	7.3 (0.3)	7.3 (0.31)	0.906	0.706
SN-1: 1,000 mg/day	7.3 (0.4)	7.2 (0.4)	7.2 (0.3)	7.4 (0.4)	7.2 (0.3)	7.3 (0.53)		
Total	7.3 (0.4)	7.3 (0.4)	7.3 (0.3)	7.4 (0.3)	7.3 (0.3)	7.3 (0.42)		
Albumin (g/dl)								
SN-1: 500 mg/day	4.7 (0.3)	4.6 (0.1)	4.8 (0.3)	4.8 (0.2)	4.6 (0.3)	4.7 (0.8)	0.345	0.884
SN-1: 1,000 mg/day	4.7 (0.3)	4.6 (0.1)	4.7 (0.3)	4.8 (0.5)	4.7 (0.3)	4.5 (0.3)		
Total	4.7 (0.3)	4.5 (0.1)	4.8 (0.3)	4.8 (0.4)	4.6 (0.3)	4.6 (0.3)		
BUN (mg/dl)								
SN-1: 500 mg/day	11.2 (3.1)	11.3 (1.8)	10.4 (1.3)	9.7 (1.8)	11.6 (2.5)	11.2 (1.9)	0.662	0.981
SN-1: 1,000 mg/day	10.4 (2.6)	10.7 (2.3)	10.9 (3.3)	11.4 (3.5)	11.1 (3.6)	11.0 (2.7)		
Total	10.8 (2.8)	11.0 (2.0)	10.7 (2.4)	10.5 (2.8)	11.4 (3.0)	11.1 (2.3)		
Creatinine (mg/dl)								
SN-1: 500 mg/day	0.71 (0.08)	0.73 (0.08)	0.79 (0.12)	0.75 (0.09)	0.84 (0.13)	0.75 (0.04)	0.158	0.677
SN-1: 1,000 mg/day	0.80 (0.13)	0.71 (0.06)	0.81 (0.11)	0.83 (0.15)	0.79 (0.13)	0.75 (0.10)		
Total	0.76 (0.11)	0.72 (0.07)	0.80 (0.11)	0.79 (0.13)	0.81 (0.13)	0.75 (0.07)		
Amylase (unit/L)								
SN-1: 500 mg/day	76.5 (33.3)	73.1 (26.5)	74.3 (19.5)	73.8 (22.2)	74.1 (25.2)	74.1 (21.0)	0.515	0.513
SN-1: 1,000 mg/day	67.5 (15.8)	65.7 (20.4)	71.8 (14.3)	66.6 (21.2)	68.0 (17.5)	64.7 (17.7)		
Total	72.0 (25.6)	69.4 (23.2)	73.1 (16.6)	70.2 (21.3)	71.0 (21.2)	69.4 (19.4)		
Sodium (mmol/L)								
SN-1: 500 mg/day	141.1 (0.8)	141.5 (1.6)	141.0 (1.0)	141.6 (0.5)	140.3 (2.1)	141.3 (1.7)	0.127	0.536
SN-1: 1,000 mg/day	141.3 (0.9)	141.5 (1.1)	140.8 (0.8)	142.1 (1.3)	140.2 (0.7)	139.2 (2.4)		
Total	141.2 (0.8)	141.5 (1.4)	140.9 (0.9)	141.8 (1.0)	140.3 (1.5)	140.3 (2.3)		
Potassium (mmol/L)								
SN-1: 500 mg/day	4.0 (0.0)	4.2 (0.2)	4.1 (0.3)	4.0 (0.2)	4.1 (0.1)	4.1 (0.2)	0.454	0.541
SN-1: 1,000 mg/day	4.2 (0.4)	4.3 (0.4)	4.3 (0.5)	4.0 (0.4)	4.2 (0.3)	4.1 (0.4)		
Total	4.1 (0.3)	4.2 (0.3)	4.2 (0.4)	4.0 (0.3)	4.1 (0.2)	4.1 (0.3)		
Chloride (mmol/L)								
SN-1: 500 mg/day	105.3 (1.1)	106.5 (1.1)	105.3 (1.5)	104.5 (0.9)	105.6 (1.4)	105.6 (0.9)	0.082	0.290
SN-1: 1,000 mg/day	104.1 (2.9)	106.1 (2.0)	105.1 (1.9)	104.5 (1.6)	105.7 (1.6)	103.1 (1.4)		
Total	104.7 (2.2)	106.3 (1.6)	105.2 (1.7)	104.5 (1.2)	105.6 (1.4)	104.3 (1.7)		
Bicarbonate (mmol/L)								
SN-1: 500 mg/day	23.0 (1.3)	23.1 (1.2)	23.0 (2.2)	23.7 (2.0)	24.1 (1.6)	21.3 (2.8)	0.166	0.341
SN-1: 1,000 mg/day	23.5 (1.9)	23.5 (1.5)	23.3 (1.5)	23.5 (1.0)	23.3 (0.5)	23.2 (1.4)		
Total	23.2 (1.6)	23.3 (1.3)	23.1 (1.8)	23.6 (1.5)	23.7 (1.2)	22.3 (2.4)		

day doses for 14 days in a total of 16 healthy volunteers. In this study, oral SN-1 extract formulation doses up to 1,000 mg/day were well-tolerated. No serious clinical or laboratory adverse events were observed in either study group. Some volunteers complained about some abnormal symptoms during drug administration in the study, as follows: headache (12%), hunger (6%), anorexia (18%), diarrhea (6%), constipation (12%), insomnia (18%) and drowsiness (18%). The most frequently reported degree of symptom was mild. Nausea and vomiting were not reported by any volunteer through out the study. It was difficult to distinguish abnormal symptoms experienced that were not related to the study drug, and study drug-related side-effects. All of the symptoms resolved at the end of drug administration. Therefore, the investigators considered the experienced symptoms as "not related to study drugs" or "unlikely to be AEs".

Laboratory monitoring showed variations each follow-up day, but no significant differences were observed among follow-up days in respect to the baseline data, and among doses of treatments. A possible explanation was that the number of volunteers in the study was small, and the groups were heterogeneous, which may have influenced the outcome of the laboratory data, as described in other studies [14-15].

In conclusion, the oral SN-1 extract formulation was well-tolerated. The results can be interpreted by the present data that the safety and blood chemistry profile are not different between the two different doses. No clinical adverse reactions or drug-related effects were observed during the study. Further investigation should clarify the pharmacokinetic profiles of oral SN-1 extract formulation for both healthy and HIV-infected subjects, as well as establish the most potent doses to inhibit enzyme HIV-1 reverse transcriptase via oral SN-1 extract formulation on HIV-infected subjects.

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