EFFICACY AND SAFETY OF ZIDOVUDINE AND ZALCITABINE COMBINED WITH A COMBINATION OF HERBS IN THE TREATMENT OF HIV-INFECTED THAI PATIENTS

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Abstract. A randomized double blind placebo controlled trial to determine the efficacy and safety of combined-herbs (SH) given with zidovudine (ZDV) and zalcitabine (ddC) for the treatment of HIV infection in Thai adults was conducted in 3 hospitals in northern Thailand during 2002 to 2003. The eligible subjects were HIV-infected Thai adults who had never received anti-retrovirals, had a Karnofski Performance Score (KPS) of ≥70, and had no opportunistic infections. The subjects were randomized to receive either a combination of ZDV 200 mg three times per day, ddC 0.75 mg three times per day, and SH 2.5 g three times per day or a combination of ZDV 200 mg three times per day, ddC 0.75 mg three times per day, and placebo 2.5 g three times per day for 24 weeks. The main outcome measures were HIV-RNA, CD4 cells, and blood chemistry profiles prior to the treatment and then every 4 weeks for 24 weeks. The baseline characteristics of 60 evaluable subjects, 40 in the SH group and 20 in the placebo group, were not significantly different. HIV RNA at week 4 and thereafter was significantly decreased from the baseline value in both groups ($p<0.001$). However, the decline in HIV RNA in the SH group was significantly more than that in the placebo group. The CD4 cells in the SH group at week 12 and thereafter were significantly increased from the baseline value. Serious adverse events in the two groups were not observed. It is concluded that an addition of SH herbs to two nucleoside reverse transcriptase inhibitors has greater antiviral activity than antiretrovirals only. The SH herbs may be an alternative for the third anti-retroviral agent in the triple drug regimen for the treatment of HIV infected patients in countries with limited resources.

INTRODUCTION

According to the official documents of the Ministry of Public Health on the Burden of Disease and Injuries in Thailand in 1999 (The Thai Working Group on Burden of Disease and Injuries, 2002), infectious diseases comprised 21% (2 million Disability Adjusted Life Years, DALYs) of the total 9.6 million DALYs. HIV/AIDS accounted for a majority of the infectious disease burden, with 1.3 million DALYs or 72% of the total infectious disease burden. It is estimated that there were more than 600,000 cases of HIV infection in the Thai population in 2003. Appropriate anti-retroviral treatment has been demonstrated to be safe, effective and efficient (Freedberg *et al,* 2001). However, most HIV-infected Thai patients have not received appropriate regimens of anti-retroviral agents, especially the protease inhibitors. The main reason for this inequity is that they cannot afford the cost of the combination of anti-retrovirals and the appropriate anti-retroviral regimens have not yet been included in any benefit schemes of health security systems in Thailand.

In order to search for medicinal herbs efficacious for the treatment of HIV infection, a research and development project on medicinal herbs for HIV infection has been established by the Department of Medical Sciences (DMSC),

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Ministry of Public Health, Thailand since 1997. In addition to screening for anti-retroviral activity of medicinal herbs grown in Thailand, a collaboration with Kunming Institute of Botany (KIB) in China was also founded in 1999. One of the herbal candidates in this joint research and development project between DMSC and KIB is a herbal formulation containing 5 herbs (SH) which has been widely used in China. The aforementioned SH herbs are *Glycyrrhiza glaba* L., *Artemisia capillaris* Thumb., *Morus alba* L., *Astragalus membranaceus* Bge., and *Carthamus tinctorius* L. The chemical compositions of these herbs have been reviewed (Tang and Eisenbrand, 1992). *In vitro* study of SH against HIV-1 was conducted by the modified Miyoshi's and Yarchoan's method (Miyoshi *et al*, 1982). It was found that each herb and the combined SH herbs had anti-retroviral activity, and their anti-retroviral activity was mediated neither via reverse transcriptase inhibition nor protease inhibition (Luo *et al,* 1998; Chen *et al,* 2002). A subacute toxicity study of SH in rats was performed by the Medicinal Plant Research Institute (MPRI), Thailand (Chavalittumrong *et al,* 2000). The suspension of SH in 1% tragacanth was given orally to Wistar rats at doses of 100, 500, and 2,500 mg/kg body weight/day (equivalent to 1-, 5-, and 25-fold the therapeutic dose in humans) for 28 days. The rats receiving 1- and 5- fold the therapeutic dose had no negative effects, but those receiving 25-fold the therapeutic dose had an elevation of liver enzymes. The phase I/II clinical trial of SH in 28 asymptomatic HIV-infected patients with CD4 >200 and HIV RNA <100,000 copies/ml was conducted at San Pa Tong Hospital, Thailand in 2000. They received only SH 5 grams per day for 3 months. It was found that 12 subjects (42.9%) had decreased HIV RNA greater than 0.5 log, but an increase in CD4 cells was not observed during the treatment period. Serious adverse events were not detected. This observation prompted us to conduct a phase III clinical trial of SH herbs as adjunctive therapy for HIV infection.

The objective of this study was to determine the efficacy and safety of SH herbs as adjunctive therapy with two nucleoside reverse transcriptase inhibitors in a randomized double blind placebo controlled fashion.

MATERIALS AND METHODS

This randomized double blind placebo controlled study was approved by the Ethics Committee of the Ministry of Public Health, Thailand. The study was conducted in one community hospital and two general hospitals in northern Thailand, namely San Pa Tong Hospital, Nakhonping Hospital and Lumphun Hospital from July 2002 to June 2003. The study subjects were Thai adults (age >20 years) with laboratory evidence of HIV-1 infection, who had never received anti-retrovirals, had a Karnofski Performance Score (KPS) of ≥70, had no opportunistic infections and signed the consent form to participate the study after being clearly informed. The subjects were randomized to the SH group or the placebo group. The subjects in the SH group received a combination of zidovudine (ZDV) 200 mg three times per day, zalcitabine (ddC) 0.75 mg three times per day, and SH 2.5 g three times per day for 24 weeks. The subjects in the placebo group received a combination of ZDV 200 mg three times per day, ddC 0.75 mg three times per day, and a placebo which was made of black glutinous rice (*Oryza sariva*) 2.5 g three times per day for 24 weeks. The subjects were scheduled for follow-up visits every four weeks. Laboratory determinations of HIV-RNA, CD4 cells, complete blood counts, renal profiles, liver profiles, fasting blood sugar and electrolytes were performed at baseline and then every 4 weeks up to 24 weeks. HIV-RNA was determined by a quantitative reverse transcriptase polymerase chain reaction assay (Amplicor HIV-1 monitor, Roche Diagnostic Systems) with a limit of detection of 400 copies per ml. The primary efficacy outcome was a reduction of plasma HIV RNA and the secondary efficacy outcome was a change in the CD4 cells from baseline. The subjects were interviewed and examined by the investigators at each visit. A comparison of the data between the two groups was performed by the Student's *t*-test or chi-square test where appropriate. The comparison of HIV RNA, CD4 cell count and laboratory profiles on different occasions over the study period was performed using repeated measured analysis of variance (ANOVA). A p-value of ≤0.05 was considered statistically significant.

RESULTS

Eighty-two eligible subjects were recruited. Forty-four subjects were assigned to the SH group and 38 subjects to the placebo group. However, only 60 cases (73.1%) had an HIV RNA test at week 12 and these subjects were evaluated for efficacy analysis. Twenty-two subjects were excluded from efficacy analysis; 10 subjects were lost to follow-up (2 cases in the SH group and 8 cases in the placebo group) and 12 subjects had adverse effects (2 cases in the SH group and 10 cases in the placebo group). The common adverse effects causing the patients to withdraw from the study were nausea, vomiting and abdominal discomfort. Among the 60 subjects available for efficacy analysis, 40 cases were in the SH group and 20 cases in the placebo group. The baseline characteristics of the subjects in the SH group and the placebo groups were not significantly different, as shown in Table 1. More than 90% of the patients were heterosexuals. Most of the subjects had a CD4 count over 200 cells/ mm^3 and HIV RNA less than 100,000 copies/ ml. Other baseline laboratory studies including complete blood counts, urine analysis, fasting blood sugar, electrolytes, renal profiles and liver profiles were not significantly different between the two groups.

Effects on HIV RNA

The mean and standard error of mean of log HIV RNA of the patients in the SH group and the placebo group at weeks 0, 4, 8, 12, 16, 20, and 24 are shown in Table 2. HIV RNA at week 4 and thereafter was significantly decreased when compared to the baseline value in both groups ($p<0.001$). However, the decline in HIV RNA in the SH group was significantly more than that in the placebo group ($p<0.001$). The percentage of patients who had a decline in HIV RNA of more than 1log, 1.5 log and 2 log at week 20 and week 24, in the SH group was significantly higher than those in the placebo group as shown in Table 3. Moreover, 3 patients in the SH group had undetectable HIV RNA at week 24, whereas none in the placebo group had undetectable HIV RNA.

Effects on CD4 cells

The mean and standard error of mean of CD4 cells in the patients in the SH group and the placebo group at weeks 0, 4, 8, 12, 16, 20, and 24 are shown in Table 4. CD4 cells at week 12 and thereafter in the SH group were significantly increased when compared to the baseline value ($p<0.05$), whereas no significant changes in CD4 cells in the placebo group during the 24 weeks of follow-up were observed.

Adverse effects

In addition to the adverse effects of gastrointestinal symptoms, as described earlier, there were no significant changes in profiles of blood cells, urine, blood glucose, renal functions, electrolytes, alkaline phosphatase and albumin

Table 1
Baseline characteristics of 60 subjects available for follow-up.

Characteristic	SH group (N = 40)	Placebo group (N = 20)	p-value
Gender- Male : Female	7:33	4:16	1
Mean age ± SD (yr)	35.6 ± 8.2	31.9 ± 8.1	0.1
Body weight ± SD (kg)	52.6 ± 9.5	51.4 ± 7.1	0.6
Karnofski Performance Score = 100	37 (92.5%)	20 (100%)	1
HIV RNA (copies/ml)			
Mean ± SE	81,590 ± 24,451	113,513 ± 31,088	0.43
Range	6,500 - 488,000	8,650 - 518,000	
HIV RNA ± SE (log copies/ml)	4.69 ± 0.07	4.79 ± 0.12	0.45
CD4 count (cells/mm^3)			
Mean ± SE	362 ± 28	430 ± 31	0.21
Range	103 - 883	64 - 1,025	

Table 2
Log HIV RNA of the patients in the SH group and the placebo group at weeks 0, 4, 8, 12, 16, 20 and 24.

	SH group (mean ± SE)	Placebo group (mean ± SE)
Week 0	4.69 ± 0.07	4.79 ± 0.12
Week 4	2.94 ± 0.12	3.82 ± 0.17
Week 8	2.92 ± 0.12	4.20 ± 0.12
Week 12	2.90 ± 0.10	4.12 ± 0.14
Week 16	2.80 ± 0.11	4.30 ± 0.14
Week 20	2.77 ± 0.11	4.18 ± 0.18
Week 24	2.65 ± 0.15	4.15 ± 0.27

Table 3
Log HIV RNA of the patients in the SH group and the placebo group at weeks 20 and 24.

	SH group (%)	Placebo group (%)	p-value
HIV RNA reduction at week 20			
≥ 1.0 log	89.7	26.3	<0.001
≥ 1.5 log	74.4	10.5	<0.001
≥ 2.0 log	41.0	10.5	0.04
HIV RNA reduction at week 24			
≥ 1.0 log	100	36.4	<0.001
≥ 1.5 log	70.8	18.2	0.011
≥ 2.0 log	54.2	9.1	0.02

in both groups of patients over the 24 weeks of observation. However, the patients receiving SH had an asymptomatic transient elevation of liver enzymes (SGOT and SGPT) during the first 8 weeks of treatment.

DISCUSSION

To our knowledge, this is the first randomized double blind placebo controlled trial to examine the effects of medicinal herbs combined with two nucleoside reverse transcriptase inhibitors on HIV-infected patients. A combination of 5 herbs (SH) was chosen due to the promising results in pre-clinical studies and phase I/II clinical studies. Since the effect of SH alone is weak, it must be used as adjunctive therapy with anti-

Table 4
CD4 cells of the patients in the SH group and the placebo group at weeks 0, 4. 8, 12, 16, 20 and 24.

	SH group (mean ± SE)	Placebo group (mean ± SE)
Week 0	362 ± 28	430 ± 51
Week 4	378 ± 29	403 ± 47
Week 8	365 ± 25	419 ± 55
Week 12	444 ± 26	483 ± 54
Week 16	452 ± 34	429 ± 52
Week 20	463 ± 32	409 ± 50
Week 24	424 ± 38	394 ± 52

retroviral drugs. The regimen of ZDV and ddC were used as the main treatment in this study because the regimen of ZDV combined with ddC or ddI was shown to decrease the HIV viral load as well as slow the progression of HIV disease and improve survival in patients (Hammer *et al,* 1996; Katzenstein *et al,* 1996); it was recommended as the standard treatment of HIV-infected patients in Thailand when the research proposal was submitted to the ethics committee in 2000. The authors did not expect to find that the patients in the placebo group had adverse effects more often than did the patients in the SH group. This finding may be due to the effect of the ingredient in the placebo that was black glutinous rice (*Oryza sariva*). This substance was chosen as a placebo because its color was very similar to that of SH. Therefore, the number of subjects who received the placebo and were suitable for efficacy analysis was less than that in the SH group. However, the baseline characteristics of the patients in both groups were not significantly different. A decline in HIV RNA at week 24 in the placebo group was only 0.6 log which is comparable to other studies which observed a decline of 0.4 to 1.6 logs at week 24 to 32 (Collier *et al,* 1996; Katzenstein *et al,* 1996; Delta Coordinating Committee and Delta Virology Committee, 1999). HIV RNA in the SH group declined approximately 2 logs from baseline value as shown in Table 2. This observation implied that this further decline in HIV RNA was due to the effect of SH. Moreover, all sub-

jects in the SH group had a decline in HIV RNA of at least 1 log, whereas only one third of the subjects in the control group did so. The CD4 cells in the SH group increased by 80 to 100 cells when compared with the baseline value, whereas CD4 cells in the placebo group at the end of the study which were not significantly different from the baseline value. These aforementioned observations from this small preliminary study imply that SH herbs are safe as well as effective in reducing HIV RNA in HIV-infected adult patients when used with two reverse transcriptase inhibitors. Since SH herbs were found to have anti-retroviral action without inhibiting reverse transcriptase or protease, SH may be an alternative for the third anti-retroviral agent in the triple drug regimen for the therapy of HIV infection in countries with limited resources. The mechanism of the anti-retroviral effect of SH and the *in vitro* activity of SH against resistant strains of HIV are being tested. The clinical trial comparing two reverse transcriptase inhibitors combined with either SH or a protease inhibitor is being planned for the treatment of asymptomatic and symptomatic HIV-infected patients. Moreover, the efficacy and safety of SH herbs for use in long-term treatment should also be evaluated.

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