

6-MONTH EVALUATION OF JINHUANG CHINESE HERBAL MEDICINE STUDY IN ASYMPTOMATIC HIV INFECTED THAIS

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Abstract. Good results of *in vitro* study of anti-HIV effects of JinHuang, a Chinese herbal medicine led to *in vivo* study of safety and efficacy among asymptomatic HIV infected individuals. It was a prospective open study of 21 asymptomatic HIV infected Thai volunteers. Twelve and 9 were female and male, respectively, with mean age of 29.24 ± 3.94 years. JinHuang preparation, 6 capsules and 2 bottles of liquid formula orally three times a day, was given on an outpatient basis initially for 6 months. Regular close monitoring and follow-up were done. The side effects reported included : increased bowel movements (81%), vague taste, and smell of drug after initiation (52%). No serious adverse event related to JinHuang was detected during study. No significant changes in terms of log viral load and CD4 count were observed after 6-months' duration. Most of the patients felt that the quality of life was better in terms of better appetite, good sleep and healthy during study participation, however, these were subjective.

INTRODUCTION

Thailand has been recognized as a high HIV prevalent country in Asia (Piot, 1998). In January 2002, 257,971 symptomatic HIV-infected Thais (23.5 per 100,000 population) were cumulatively reported by Ministry of Public Health of Thailand (Anonymous, 2002), increasing from HIV/AIDS prevalence reported the previous year. The mortality rate from HIV/AIDS was in the first rank among leading national infectious diseases, implying that this infection will continue to be a major health problem in Thailand. From previous reports, AIDS mortality mostly affected the generation aged 20-35 years (Anonymous, 2002) that would be important for country development. However, there was a decreasing number of additional new HIV/AIDS diagnosed during 1996-2000 (30,000 cases) compared to 800,000 cases estimated in 1995 by the National

Economic and Social Development Board (NESDB) (Kunanusont *et al*, 1999). This trend of decreasing HIV/AIDS incidence since 1995 implies that the HIV control program effected by the Thai government and public sponsors really worked (Phoolcharoen, 1998). Inevitably, an increased number of symptomatic or AIDS patients is expected in the next few years due to natural progression rate estimated around 6.8% per year (Sirivichayakul *et al*, 1992).

Effective antiretroviral drugs and regimens have been published and accepted worldwide for specific treatment and subsequent life span prolongation. However, these agents are not available to most of patients due to their high cost, common adverse effects, in company with uncertain period of their effective use. Therefore, the optimal goal of treatment can not be accomplished. Thailand started the antiretroviral supply program primarily for low income groups in 1992. The budget has increased inversely with decreased coverage because of its high price (Kunanusont *et al*, 1999). Now it seems that only the rich can afford HAART regimens of antiretroviral therapy. Prophylactic HIV vaccines

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would be a new hope for global HIV incidence reduction and choice of prevention which unfortunately is now under development.

Besides low risk behavioral practices encouragement, the only option for unaffordable ones is complementary remedies. Many kinds of complementary treatments such as herbal medicine are often widely used by HIV infected individuals (Wu *et al*, 2001), yet little is known about their effectiveness in scientific tests (Ozsoy and Ernst, 1999). Chinese herbal medicine has been introduced for chronic illness for many centuries especially in Asia (Vickers and Zollman, 1999). Since the onset of the AIDS pandemic, there have been many reports demonstrating HIV-1 inhibitory effect *in vitro* (Ono *et al*, 1990; Li *et al*, 1993; Chag *et al*, 1995; Collins *et al*, 1997; Yamasaki *et al*, 1998; Weber *et al*, 1999; Au *et al*, 2001; Min *et al*, 2001), but mostly in non-English journals. JinHuang is a Chinese herbal medicine with evident safety and efficacy both *in vitro* and in animal models. In addition, a phase I clinical trial in 5 Chinese HIV/AIDS patients demonstrated promising effects of significant viral load decrease (unpublished data). This trial in asymptomatic HIV infected Thais aimed to evaluate safety and efficacy of JinHuang "Chinese Herbal Medicine". Analysis at 6-months duration could help in predicting the possibility of continuing this study if it is proved to be safe and tolerable with a trend of effective anti-HIV insults.

MATERIALS AND METHODS

This open prospective clinical trial was done at the Clinical Infectious Disease Research Unit, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University with collaboration of Bamrasnaradura Hospital. The Protocol was approved by The National Ethical Committee on Clinical Research, Thailand.

Subjects

Twenty-five HIV infected Thais aged 20-60 years who voluntarily signed consent forms during 23 April to 26 July, 2001 were screened by history taking, physical examination and laboratory investigations including complete blood count, serum creatinine, electrolyte, liver func-

tion tests, urine analysis, chest roentgenography, electrocardiography. Only 21 asymptomatic HIV infected Thais who proved to show no serious organ dysfunction in renal, hepatic, cardiac, pulmonary and hematological systems were enrolled into this study. All patients had base-line CD4 counts $\geq 200/\text{mm}^3$ and quantitative plasma HIV RNA tests both of which were obtained 2 times, 2 weeks apart prior to JinHuang administration. Two volunteers who had CD4 counts less than $200/\text{mm}^3$ (average 152.5 and 159.5 cells/ mm^3) were enrolled with exemption on agreement of the sponsor. To assure that JinHuang was not used in pregnancy, all female participants agreed to use acceptable effective contraception and have negative pregnancy test results before enrollment and throughout the study period. Education of risk reduction and counseling were done at every visit. All volunteers revealed the status of well being (Karnofsky score ≥ 70) to attain the participation of the study. They understood well about the basic knowledge of HIV and were aware of the need to adhere to the scheduled regimen of herbal medicinal drug and give sufficient time for regular visits every week for the first 1 month and every 2 weeks for the rest of time.

Drugs and doses

JinHuang comprises Radix Curcumae, artificial Calculus Bovis, Margarita, Radix Noto-ginseng, Radix Aucklandiae, R. officinale Baill, Sichuan fritillary bulb and Borneolum. Six capsules of JinHuang Capsule (450 mg/capsule) and 2 bottles of JinHuang Oral (0.1 mg/ 5 ml/bottle) were prescribed three times immediately after meals everyday for 6 months. Both JinHuang preparations were manufactured and supplied by HuaTai Pharmaceutical Co Ltd, Shantou High-Tech Zone, China.

Monitoring and evaluations

Safety issue was the important primary endpoint of this study. To alleviate the serious drug allergy that possibly occur, a one day observation after initiation of the first dose was done in all cases and 24-hour call line was available promptly for any urgent events throughout the study. Daily visits for one week, then weekly for the next 4 weeks, and biweekly thereafter were required. Diary cards were given to the patients

to record any adverse events occurred and time drugs taken during the study. Blood drawn for complete blood count and urine analysis were scheduled at baseline before the study and every month during the study. Liver function tests, serum creatinine were checked every 2 months and electrocardiogram was monitored every 6 months during the study. The efficacy of JinHuang was determined by comparison of the base-line CD4 counts and viral load test before enrollment, and every 3 months of follow-up during the study. An increase in CD4 cell numbers by statistical analysis and/or decreased log reduction of viral load of ≥ 0.5 would acceptably indicate effective changes resulting from anti-HIV effect of JinHuang. In addition, the quality of life and psychological attitude were evaluated regularly by Body Mass Index measurement, questionnaires and periodic counseling.

Statistics analysis

CD4 cell count and log viral load change during study were assessed with Mann-Whitney U test by Strata Software serial 1960511686 while other parameters of safety index were compared during the study. Body Mass Index calculation and subjective evaluation were done at 6 months of the study. Assessment of quality of life from the questionnaires was presented as proportion and frequency for analysis.

RESULTS

Background characteristics

All 21 asymptomatic HIV infected Thais with mean \pm SD aged of 29.24 ± 3.94 years were naïve to antiretroviral treatment prior to enrollment. 23.8%, 38.1% and 38.1% were 21-25, 26-30 and 31-35 years old, respectively. 57.1% were females, the majority were single (47.6%). All volunteers reported HIV infection from sexual practices. History of blood transfusion was noted only about 9.5%. Monogamy with a risky sexual partner, which was the most common risk behavior in this group (62%), was found mostly in women. Having sex with multiple partners and commercial sex workers were 33% and 5%, respectively; homosexual men were 9.5%. No underlying disease or history of opportunistic infection was detected from history and physical

examination. Karnofsky scores were 100 in all cases. Twenty patients adhered to scheduled treatment and follow-up visits. One patient withdrew her consent at the 6-month visit due to travelling inconvenience that was not related to the drug.

Safety profile

No severe adverse event relevant to this drug was detected. One female volunteer experienced small amount of abnormal vaginal bleeding for 5 days after 1 week of JinHuang administration. She was admitted but no abnormality was revealed on physical examination. Her symptom spontaneously resolved. No skin or systemic allergic symptom was detected. Adverse events within the first 6-month period that were possibly related to this drug were a slight increase of bowel movement without perception of life disturbance and treatment needed (81%), vague taste and smell of the drug (52.4%), dizziness (9.5%), intermittent itching sensation (9.5%), and transient breast congestion (4.76%). Most patients experienced those adverse events within 2 weeks after initiation of the study. The symptoms were either self-limited within 2 months of initiation or intermittently occurred except increased bowel movement persistently continued through the study in the ones who experienced. The adverse events were well tolerated and not required any medical treatment. None stopped the drug because of adverse events. There was no statistically significant change of any safety parameters, hemoglobin level, white blood count, platelets count, liver enzyme level, creatinine level and Karnofsky score during 6-month period of this study as shown in Table 1.

Efficacy assessment

Median (range) of CD4 count at baseline (compiled from group of mean CD₄ level of first 2 visits - screening and first visits in each case) before treatment was 369.5 cells/mm³ (152.5 - 714 cells/mm³). While mean CD4 count at 3 and 6 month interval were 367 cells/mm³ (135-626 cells/mm³) and 324 cells/mm³ (119-532 cells/mm³), respectively (Fig 1). Median viral load at the beginning of treatment and at 3, 6 months after treatment were demonstrated in Table 2. There was no statistically significant change of CD4 cell count ($p=0.825$) and viral load ($p=0.814$) between

Table 1
Demonstration of safety parameters monitored during study.

Parameter	Median (Min-Max)		
	Base line	3 Months	6 Months
Hemoglobin (g/dl)	14 (10.4-17)	13.3 (11.3-16.6)	13.4 (10.5-16.2)
Hematocrit (%)	39.6 (33.3-48.3)	40.6 (33.9-49.4)	39.9 (31.3-49.4)
WBC ($\times 10^3/\text{mm}^3$)	5.45 (3.4-8.99)	5.06 (3.5-10.8)	5.02 (3.72-8.52)
Platelets ($\times 10^6/\text{mm}^3$)	2.24 (1.48-2.91)	2.26 (0.78-3.07)	2.53 (1.09-3.41)
Creatinine (mg%)	0.93 (0.75-1.39)	0.93 (0.66-1.43)	1.13 (0.79-1.6)
ALT ^a (U/ml)	20 (5-37)	11 ^a (7-33)	15 (9-34)
AST ^a (U/ml)	20 (11-33)	17 ^a (9-31)	18.5 (10-25)
Albumin (mg/dl)	4.4 (3.8-4.8)	4.3 (3.7-4.8)	4.25 (3.7-4.6)
Karnovsky score	100 (90-100)	100 (100)	100 (100)

^aALT, AST, albumin were tested every 2 months during study and level at 4 - month visit were demonstrated here.

Table 2
Demonstration of efficacy parameters during study.

Parameter	Median (Min-Max)		
	Base line ^a	3 Months	6 Months
CD4 count (cell/mm ³)	369.5 (152.5-714)	367 (135-626)	324 (119-532)
VL ($\times 10^3$ copies/ml)	35.29 (0.46-409.6)	25.49 (0.3-306.31)	35.4 (0.33-260)
Log VL	4.51 (2.67-5.41)	4.41 (2.53-5.49)	4.54 (2.53-5.41)
BMI (kg/m ²)	20.57 (17.6-35.5)	21 (17.22-24.91)	20.45 (17.2-26)

^aCD4, VL, log VL at baseline were calculated as mean parameters from 2 times measurement.

the beginning and at 6 month of treatment. There was also no log reduction in the accepted range of viral load with no statistically significant change ($p=0.2$) at 6 month of treatment. In subgroup of patients who had viral load more than 50,000 copies/ml before enrollment, the significant reduction of viral load was found ($p=0.038$) as shown in Fig 2. However, no significant change in log reduction of viral load was shown among this subgroup. Karnofsky scores of 100 at 3 and 6 month of treatment in all patients were not changed from baseline. Most of the patients reported that their quality of life was better at 6-month evaluation in terms of better appetite, good sleep and healthy. The mean of Body Mass Index (BMI) in the subgroup of initial viral load $\geq 50,000$ copies/ml increased slightly at 6 months (Fig 3), but was not statistically significant.

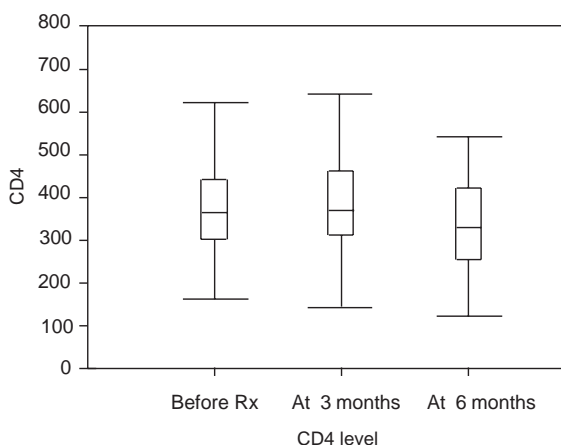


Fig 1—Median of CD4 cell count (cell/mm³) during treatment (N=21).

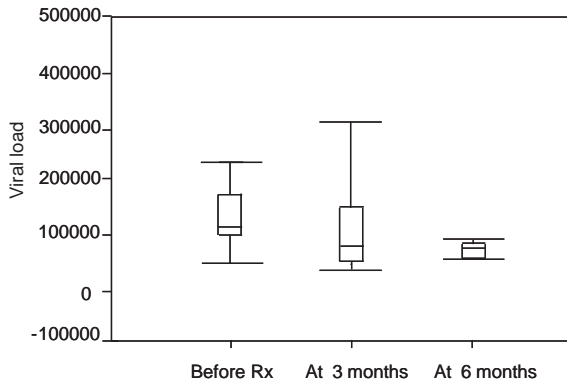


Fig 2—Change in viral load during study in subgroup VL>50,000 copies/ml (N=7).

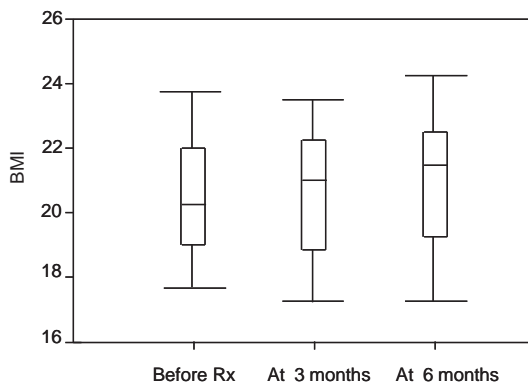


Fig 3—Body Mass Index (BMI) change during treatment in subgroup (VL>50,000 copies/ml) (N=7).

DISCUSSION

HIV/AIDS is an important health problem in Thailand by virtue of its impact on high infection rate and mortality rate reported by Thai MOPH. The common age of HIV infection developing AIDS was mainly 25-29 years old. Our patients' mean age was consistent with that peak age prevalence in Thailand. The risk of getting HIV infection in Thailand now is changing from previously mentioned risk exposure of multiple sexual partners or commercial sex workers to the exposure of single risky sexual partners. As in this study, most patients were monogamous with single sexual partner or were housewives, whose sexual practices were less risky. Although the sample size in this study was too small, the findings should raise concern of HIV transmission

among married couples or ordinary people due to unawareness of the status of their sexual partners.

JinHuang, a Chinese herbal medicine, was tested for adverse effects and efficacy in animals infected with SIVmac251 during October, 1998-June, 1999. It demonstrated viral inhibition in the acute phase of infection and restored damage of immunologic CD4 cells (Huaiyan S *et al*, unpublished data). For adverse affects, the prospective controlled trial in Rhesus models during September, 1998-December, 1999 at ZhongShan Medical University showed no difference in weight gain and hematological, hepatic and renal functions between the treatment group and the controlled group. Autopsy findings in major organs also could not demonstrated any abnormalities (Xigu C *et al*, unpublished data). The recent phase I/II open study in HIV/AIDS patients conducted in the Infectious Disease Unit of Beijing Youan Hospital demonstrated no significant adverse effect in 5 Chinese men and the increase of CD4 cell count with half decreased viral load in some cases were also evident (Lianzhi X and Ke Z, unpublished data)

After 6-month evaluation of this study, there was no evidence of beneficial effects on HIV inhibition and increase of CD4 cell count from JinHuang similar to the findings of other herbal drugs trials previously performed (Burack *et al*, 1996; Weber *et al*, 1999). In subgroup with viral load > 50,000 at the beginning of the study, though there was a statistically significant decrease in viral load, the low number of subjects (N=7) involved, and better log viral load parameter could not demonstrate any significant difference. Prudent interpretation of this finding should be advised, as further verification will need to be done at the 12-month study term. Another herbal medicine also has a promising result; a pilot study of qian-kun-nin (Zhan *et al*, 2000) demonstrated a significant decrease of viral load and increase of CD4 cell count. The mild degree of increased bowel movement commonly found in this study was also mentioned in another herbal medicine trial (Weber *et al*, 1999) and can possibly be a common side effect resulting from herbal medicine. Expectedly, more than half of HIV-infected patients in this study reported this bowel symp-

tom. Assessment of well being which was mentioned as another concerned objective (Burack *et al.*, 1996; Weber *et al.*, 1999) showed that most of the patients reported that the quality of life during the study participation was better than before. They claimed that the perceptions of specific treatment that they have never had before in this study would cause the expectancy of better outcome and psychological improvement. However, good and friendly relationship among patients and our staff can be placebo effects in quality of life assessment. The evident tolerability and safety without clinical deterioration of immunity such as opportunistic infections during the study convinces us to continue this study for longer duration up to 1 year to evaluate the efficacy of long term drug administration.

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REFERENCES

- Anonymous. *Monthly Epidemiol Surv Rep* 2002; 33: 27-35.
- Au TK, Lam TL, Ng TB, Fong WP, Wan DC. A comparison of HIV-1 intergrase inhibition by aqueous and methanol extracts of Chinese medical herbs. *Life Sci* 2001; 68: 1687-94.
- Burack JH, Cohen MR, Hahn JA, Abrams DI. Pilot randomized controlled trial of Chinese herbal treatment for HIV-associated symptoms. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996; 12: 386-93.
- Chang CW, Lin MT, Lee SS, Liu KC, Hsu FL, Lin JY. Differential inhibition of reverse transcriptase and cellular DNA polymerase- α activities by lignans isolated from Chinese herbs, *Phyllanthus myrtifolius* Moon and tannins from *Lonicera japonica* thumb and *Castanopsis hystrix*. *Antiviral Res* 1995; 25: 367-74.
- Collins RA, Ng TB, Fong WP, Wan CC, Yeung HW. A comparison of human immunodeficiency virus type 1 inhibition by partially purified aqueous extracts of Chinese medicinal herbs. *Life Sci* 1997; 60: 345-51.
- Kunanusont C, Phoolcharoen W, Bodaramik Y. Evolution of medical services for HIV/AIDS in Thailand. *J Med Assoc Thai* 1999; 82: 425-34.
- Li BQ, Fu T, Yan YD, Baylor NW, Ruscetti FW, Kung HF. Inhibition of HIV infection by baicalin-a flavonoid compound purified from Chinese herbal medicine. *Cell Mol Biol Res* 1993; 39: 119-24.
- Min BS, Kim YH, Tomiyama M, *et al.* Inhibitory effects of Korean plants on HIV-1 activities. *Phytother Res* 2001; 15: 481-6.
- Ono K, Nakane H, Fukushima M, Chermann JC, Barre-Sinoussi F. Differential inhibition of the activities of reverse transcriptase and various cellular DNA polymerase by a traditional Kampo drug, shosaiko-to. *Biomed Pharmacother* 1990; 44: 13-6.
- Ozsoy M, Ernst E. How effective are complementary therapies for HIV and AIDS? - A systematic review. *Int J STD AIDS* 1999; 10: 629-35.
- Phoolcharoen W. HIV/AIDS prevention in Thailand: Success and challenges. *Science* 1998; 280: 1873-4.
- Piot P. The science of AIDS: a tale of two worlds. *Science* 1998; 280: 1844-5.
- Sirivichayakul S, Phanuphak P, Hanvanich M, Ruxruntham K, Panmoung W, Thanyanon W. Clinical correlation of the immunological markers of HIV infection in individuals from Thailand. *AIDS* 1992; 6: 393-7.
- Vickers A, Zollman C. Herbal medicine. ABC of complementary medicine. *Br Med J* 1999; 319: 1050-3.
- Weber R, Christen L, Loy M, *et al.* Randomized, placebo-controlled trial of Chinese herb therapy for HIV-1-infected individuals. *J Acquir Immune Defic Syndr* 1999; 22: 55-64.
- Wu JA, Attele AS, Zhang L, Yuan CS. Anti-HIV activity of medicinal herbs: usage and potential development. *Am J Chin Med* 2001; 29: 69-81.
- Yamasaki K, Nakano M, Kawahata T, *et al.* Anti-HIV-1 activity of herbs in Labiatae. *Biol Pharm Bull* 1998; 21: 829-33.
- Zhan L, Yue ST, Xue YX, Attele AS, Yuan CS. Effects of qian-kun-nin, a Chinese herbal medicine formulation, on HIV positive subjects: a pilot study. *Am J Chin Med* 2000; 28: 305-12.