

## Immunomodulatory Effects of a Traditional Chinese Medicine with Potential Antiviral Activity: A Self-Control Study

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**Abstract:** Traditional Chinese medicine (TCM) has been used for prevention and treatment of severe acute respiratory syndrome (SARS) in Hong Kong during the outbreak in spring 2003. We investigated the immunomodulating effects of an innovative TCM regimen derived from two herbal formulas (*Sang Ju Yin* and *Yu Ping Feng San*) for treating febrile diseases. Thirty-seven healthy volunteers were given the oral TCM regimen daily for 14 days. Peripheral venous blood samples were taken on days 0, 15 and 29 for hematology, biochemistry and immunology tests, including the measurement of blood lymphocyte subsets and plasma T-helper lymphocyte types 1 and 2 cytokines and receptor. After 3 months, 23 of the volunteers participated in a control study without TCM treatment for the same time course of blood tests. Two volunteers withdrew on day 2, due to headache and dizziness. All others remained well without any side effects. No participants showed significant changes in their blood test results, except that the T-lymphocyte CD4/CD8 ratio increased significantly from  $1.31 \pm 0.50$  (mean  $\pm$  SD) on day 0 to  $1.41 \pm 0.63$  on day 15 ( $p < 0.02$ ), and reduced to  $1.32 \pm 0.47$  on day 29 ( $p < 0.05$ ). In the control study, there were no changes in the CD4/CD8 ratio. The transient increase in CD4/CD8 ratio was likely due to the TCM intake. We postulate that the administration of the innovative TCM may have beneficial immunomodulatory effects for preventing viral infections including SARS.

**Keywords:** SARS; Blood Lymphocytes; CD4; CD8; Traditional Chinese Medicine; *Folium isatidis*; *Radix astragali*; *Folium mori*.

## Introduction

During its 2003 outbreak in Hong Kong, severe acute respiratory syndrome (SARS) caused an immense anxiety in our society and among frontline healthcare workers because of its contagiousness, rapid progression and high mortality rate. Locally, there were 1755 infected cases with 299 deaths, accumulating to 17% mortality (Lam *et al.*, 2004). Worldwide, the affected rate for medical staff was as high as 22% (WHO, 2003). SARS is a new viral disease with no established therapy and proven cure (Lee *et al.*, 2003; Tomlinson and Cockram, 2003). In Hong Kong, where traditional Chinese medicine (TCM) is commonly used for preventive health care and treatment of minor ailments (Wong *et al.*, 1997), herbs such as *Radix isatidis* (Ban-lan-gen) were used for prophylaxis of SARS with unknown efficacy.

We investigated the mid-term immunomodulating effects of an innovative TCM preparation that was derived from two renowned centuries-old herbal formulas: *Sang Ju Yin* and *Yu Ping Feng San*. *Sang Ju Yin* was first found in the medical book *Wan Bing Tiao Bian* of the Qing dynasty, about 400 years ago. *Yu Ping Feng San* was described even earlier in the Song dynasty of the 10th century in *Shi Yi De Xiao Fang*, an ancient TCM textbook. In China, both formulas have been used popularly for prevention and treatment of “wan bing,” a febrile disease with early presentations similar to SARS and many other respiratory viral infections. According to TCM practitioners, the early “wan bing” symptoms may manifest as “cold,” “heat” and “humidity.” “Cold” refers to the feeling of chilliness; “heat” to feverishness, with or without increase in body temperature; and “humidity” to poor appetite, bowel disturbance, feeling unwell and fatigue. They include chills, fever, myalgia and diarrhea as manifested in SARS, influenza and viral pneumonia (Choi *et al.*, 2003).

## Materials and Methods

### TCM

Our formula contained 12 commonly used herbs: *Folium mori*, *Flos chrysanthemi*, *Semen armeniacae amarum*, *Fructus forsythiae*, *Herba menthae*, *Radix platycodonis*, *Radix glycyrrhizae*, *Rhizoma phragmitis*, *Radix scutellariae*, *Folium isatidis*, *Radix astragali* and *Radix saposhnikoviae*. It was decocted according to the Good Manufacturing Practice Standard (GMP) by simmering, preparing as lyophilized granules in sacs and administering as a drink after reconstitution in hot water (Chinese Medicine Industry Development Centre, Hong Kong Institute of Vocational Education, Hong Kong). Before the clinical trial, the TCM granules were tested for heavy metals, microbial and residual pesticides (Centre for Clinical Trials on Chinese Medicine, Institute of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong). All test results met the safety standards in Hong Kong.

### *Study Subjects and Blood Samples*

We recruited 37 healthy staff members from the Prince of Wales Hospital and the Chinese University of Hong Kong. On day 0, a 3 ml EDTA and a 5 ml heparinized peripheral venous blood samples were collected from each volunteer, who then consumed 1 sac (5 g) of the TCM granules per day for 14 days. On day 15 when TCM consumption had been completed and on day 29 when TCM had been stopped for 14 days, blood samples were taken again from all study subjects. Each subject was also requested to fill out a "SF-36 Health Survey" questionnaire (Lam *et al.*, 1998) on days 0, 15 and 29 for general health assessment. After 3 months, all study subjects were recalled and 23 agreed to participate in a control study. No TCM was given for these 23 subjects throughout the study period, but blood samples were collected on days 0, 15 and 29 for the same blood tests. This clinical trial was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong. Written informed consent was obtained from each participant before starting the TCM regimen.

All blood samples were analyzed for hematology, biochemistry and immunology parameters. Complete blood count and differential white blood cell count were performed on a Coulter Gen-S Hematology Analyzer (Beckman Coulter, CA, USA). Renal function test (plasma sodium, potassium, urea and creatinine), liver function test (total protein, albumin, bilirubin, alkaline phosphatase and alanine transaminase), lactic dehydrogenase and creatinine kinase were determined on a Modular Analytics DP System (Roche Diagnostics, Basel, Switzerland). Cellular immunity was evaluated by peripheral blood lymphocyte-subset analysis (B-lymphocytes, CD4 T-helper (Th) lymphocytes, CD8 T-suppressor (Ts) lymphocytes, cytotoxic T-lymphocytes (CTL) and natural killer (NK) cells), using flow cytometry (MultiTEST IMK Kit and FASCalibur flow cytometer, BD Biosciences, CA, USA). Plasma proinflammatory cytokine interleukin (IL)-18, Th type 1 (Th1) cytokine tumor necrosis factor alpha (TNF $\alpha$ ), Th type 2 (Th 2) cytokine IL-4 and soluble IL-2 receptor (sIL-2R) were measured by solid phase enzyme-linked immunosorbent assay (Diacclone Research Corp, Besancon, France).

### *Statistical Analysis*

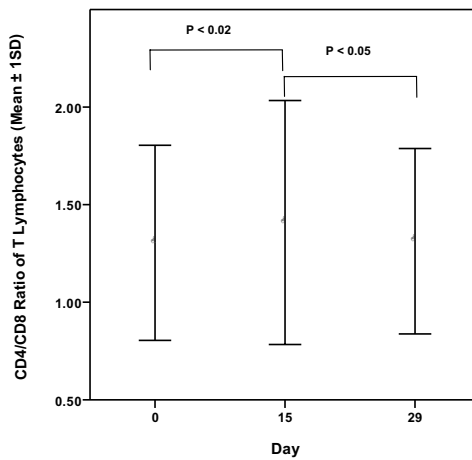
All data were expressed as mean  $\pm$  SD. Paired t-test was used for statistical analysis. A probability  $p < 0.05$  was considered significant.

## **Results**

Two of the 37 study subjects withdrew from the study on day 2 because of complaints of headache and dizziness, apparently due to taking the TCM. The remaining 35 subjects (18 males and 17 females; age  $38 \pm 10$  years, range 24–57) were well with no side effects throughout the study period. None of them experienced any symptoms of "cold," "heat"

and “humidity,” according to TCM practice. No derangements in their biochemistry and hematology tests were detected. No significant changes were observed in the absolute count and the percentage of the lymphocyte subsets ( $p > 0.05$ ). However, the CD4/CD8 ratio of T-lymphocytes increased significantly from  $1.31 \pm 0.50$  on day 0 to  $1.41 \pm 0.63$  on day 15 ( $p < 0.02$ ). On day 29, it decreased to  $1.32 \pm 0.47$  ( $p < 0.05$ , Fig. 1A). There were no sex differences in these changes. Plasma IL-18, TNF $\alpha$ , IL-4 and sIL-2R showed no significant changes (all  $p > 0.05$ ).

(A)



(B)

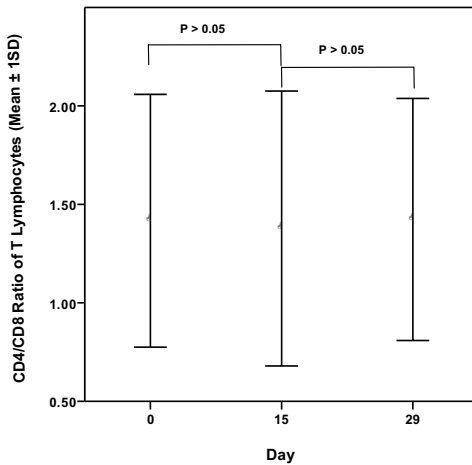


Figure 1. CD4/CD8 ratio (mean  $\pm$  SD) of T-lymphocytes on days 0, 15 and 29 in which 35 subjects took TCM from days 0 to 14 and stopped from days 15 to 28. (A) Significant increase ( $p < 0.02$ ) was observed on day 15. In the control study (B), 23 of the TCM study subjects repeated the blood testing protocol after 3 months without taking TCM.

The initial design of our study, conducted an anxious period during the peak of the SARS epidemic, included a pre- and post-study, in which no placebo controls were included. To investigate whether such transient increase in the CD4/CD8 ratio was due to the TCM intake, we recalled all 35 subjects for a control study after 3 months. Twenty-three (12 males and 11 females; age  $37 \pm 11$  years, range 24–57) agreed to participate. There were no significant changes in the CD4/CD8 ratio throughout the period of the control study (all  $p > 0.05$ , Fig. 1B). All biochemistry, hematology and immunology test results remained unchanged and were within their normal ranges.

## Discussion

TCM and Western medicine are two very different entities. Western medicine, often prescribed in the form of a single active drug with known pharmacokinetic and pharmacodynamic properties, toxicity and adverse effects, is usually efficient in the treatment of acute diseases. It is also used frequently in a combination of two or more drugs, such as the use of an antiviral drug (ribavirin), combined with anti-inflammatory steroids for the treatment of SARS patients (Zhaori, 2003). For treating acquired immune deficiency syndrome (AIDS), the highly active anti-retroviral therapy (HAART) combines three to four protease and reverse transcriptase inhibitors to forestall the dynamic HIV-1 infection (Ho, 1995; Finzi *et al.*, 1997). Western medicine usually targets directly on a disease, rather than for prophylaxis or treatment. TCM accentuates on maintaining and restoring balance, and harmony of the body. It enhances the body's defense against development of disease, or to improve prognosis. TCM is usually prescribed in formulas containing several medicines: the "chief" or the king (*Zhing*) medicine, to provide the strongest therapeutic action for balancing the disharmony; the "adjuvant" or "minister" (*Chen*) medicine to assist the "chief" medicine in its therapeutic actions for a lesser aspect of the disharmony; the "assistant" (*Zhou*) medicine to assist the "adjuvant" medicine to treat a much lesser aspect of the disharmony, and the "guider" (*Shi*) medicine to either reduce the toxicity of the medicines in the formula, or guide the formula to the targeted organ or region of the body (Yen, 1992; Cheng, 2000). Most TCM preparations have been used for decades if not centuries with minor alterations in ingredients and simmering methods by expert practitioners applying their practical experience. Their safety has well maintained the test of time. Meanwhile, an increasing number of randomized controlled clinical trials have been conducted to provide evidence of the efficacy of TCM in the treatment of a variety of diseases (Kam and Liew, 2002). TCM is considered to be more appropriate in maintaining health and balance for preventing illnesses from occurring and, because of few known adverse effects, for the treatment of chronic diseases. It has been estimated that the number of reported severe adverse effects of TCM is relatively low, compared with Western medicine (Hosbach *et al.*, 2003). In our study of 37 subjects, we encountered only two (5.4%) participants who developed headache and dizziness after taking the TCM. Nevertheless, those were mild adverse effects that subsided with termination of drug intake. For laboratory monitoring of adverse effects, we performed blood tests commonly used for clinical trials of Western medicine. Hematological parameters, renal function test, liver function test and muscle

enzymes of all the remaining study subjects were within normal ranges throughout the entire study period. Thus, consumption of the TCM preparation should not lead to any adverse effects on blood cells, kidneys, liver and muscle of the body.

During the 2003 outbreak in Hong Kong, when Western medicine was solely used for treating SARS, the death rate was 17% (299 deaths among 1755 affected patients). Similar death rates of 17% (43 among 251 patients) and 14% (33/238) were recorded in Canada and Singapore, respectively (WHO, 2003). All three countries used similar combination therapy of ribavirin and anti-inflammatory steroids for treatment of SARS. In Mainland China, where more than 50% SARS patients were treated with integrated Western and Chinese medicine (Jia and Gao, 2003), the death rate was only 7%, 349 deaths among 5327 affected patients in the Guangdong province (Cyranoski, 2003). Thus, there is a proposition that the relatively low death rate of SARS patients in southern China was likely due to the use of TCM for treatment (Jia and Gao, 2003). Similar findings were reported by Beijing's You-an Hospital in an international Anti-SARS forum held in Beijing in June 2003. A markedly higher death rate of 47.4% was encountered in severely ill patients treated with Western medicine alone, compared to 15.4% among similar patients treated with integrated Western and Chinese medicine (Jia and Gao, 2003). However, these authors commented that it was unlikely that TCM could be used for prevention of SARS for the frontline medical staff (Jia and Gao, 2003).

In this study, we evaluated the immunomodulatory effects of an innovative TCM preparation with potential antiviral activity. Due to the practice of Western medicine in hospitals in Hong Kong, we were unable to study our TCM preparation on SARS patients. In our study of healthy volunteers, CD4/CD8 ratio of T-lymphocytes was significantly increased after taking the TCM regimen for 14 days. Since no such increase was observed after TCM consumption was stopped (day 29) as well as during the entire control study period. The transient increase was likely due to the TCM intake. CD4 and CD8 are surface markers on T-lymphocytes, with CD4 being expressed on the T-helper (Th) lymphocytes, and CD8 on the T-suppressor and cytotoxic T-lymphocytes. Both Th and suppressor/cytotoxic T-cells are subsets of T-lymphocytes that mature in the thymus. CD4 Th lymphocytes mediate immune response by producing cytokines that activate other cells of the immune system. Th cells are subdivided into Th1 or Th2 cells according to the cytokines produced. Th1 cells secrete cytokines, including interleukin 2 (IL-2), tumor necrosis factor (TNF $\alpha$  and TNF $\beta$ ) and interferon  $\gamma$  (IFN $\gamma$ ), that mainly promote cell-mediated immunity through activation of cytotoxic T-cells and macrophages. Th2 cells secrete cytokines, such as IL-4, IL-5, IL-10 and IL-13, that mainly promote humoral immunity through stimulation of antibody production by B-lymphocytes. Cytotoxic T-lymphocytes kill pathogen-infected or malignant cells, while suppressor T-lymphocytes inhibit or limit immune responses. CD4 T-lymphocyte count and CD4/CD8 ratio are often used to assess the status of the immune system. CD4/CD8 ratio had been found to increase concomitantly, with tumor regression in renal cell carcinoma patients treated with interferon-based therapy (Hernberg *et al.*, 1997). In this study, the increased CD4/CD8 ratio reflected a stimulated immune system mediated by the interferon treatment. On the contrary, in HIV-1 infection, the decrease in CD4 count and CD4/CD8 ratio lead to profound immunodeficiency and suppressed cellular immunity.

In AIDS patients, the CD4 count is usually < 200 cells/ $\mu$ l in blood and the CD4/CD8 ratio decreases to lower than 1 (Ameisen and Capron, 1991; CDC, 1992). A normal healthy individual exhibits a blood CD4 count of 600–1000 cells/ $\mu$ l and a CD4/CD8 ratio >1. In our study, although we did not encounter significant changes in the CD4 and CD8 absolute counts upon TCM treatment, the significantly increased CD4/CD8 ratio suggested that there were more Th cells than cytotoxic/suppressor T-cells, and thus a stimulated cell-mediated immunity. We postulate that administration of our innovative TCM may have beneficial immunomodulatory effects for potential prevention of viral infection including SARS. Our clinical observation on the preventive effect of this TCM against SARS among 3000+ hospital workers (Liu *et al.*, 2004) indicated that none of them who took the preventive regime was infected with SARS during the trial period. In contrast, 0.4% of the hospital workers not taking the TCM were infected. In addition to soluble IL-2 receptor, plasma proinflammatory cytokine IL-18, Th1 cytokine TNF $\alpha$ , and Th2 cytokine IL-4 were retrospectively measured. This was aimed at a more comprehensive assessment of immunomodulatory effects by investigating representative cytokine of inflammation (Wong *et al.*, 2002) and signature cytokines for activation of the Th1 and Th2 pathways (Kawakami, 2003; Jarnicki and Fallon, 2003). We are not surprised that no significant changes were observed, since these plasma tests are less sensitive than lymphocyte subset analysis, and in any case, none of these healthy subjects had signs or symptoms of infection for causing cytokine changes. However, for a complete and more sensitive assessment of the immunomodulatory effects of TCM, in the future, we shall include the lymphocyte stimulation test, in which the peripheral blood mononuclear cells (PBMC) are subjected to T- and B-lymphocyte stimulation, using phytohaemagglutinin plus bacterial lipopolysaccharide for measurement of any elevation in the genetic and protein expression of cytokines and chemokines (Wong *et al.*, 2004). This would be the most sensitive test for any improvement in cellular and humoral immune potentials in healthy subjects on TCM trials, who do not have any active disease.

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