

KP Fung 馮國培  
 PC Leung 梁秉中  
 KWS Tsui 徐國榮  
 CCD Wan 溫志昌  
 KB Wong 黃錦波  
 MYM Waye 韋妙宜  
 WNS Au 區詠娥  
 CK Wong 黃振國  
 WKC Lam 林偉基  
 BSC Lau 劉碧珊

# Immunomodulatory activities of the herbal formula Kwan Du Bu Fei Dang in healthy subjects: a randomised, double-blind, placebo-controlled study

## Introduction

Severe acute respiratory syndrome (SARS) was a life-threatening form of pneumonia caused by SARS-coronavirus (SARS-CoV). Symptoms included cough, high fever and headache that could progress to interstitial infiltrates in the lung. From late 2002 to mid-2003, SARS affected more than 8000 persons worldwide, mostly in China. Due to the absence of definitive therapeutic western medicines, agents active against this disease are still needed.

The herbal formula Kwan Du Bu Fei Dang (抗毒補肺湯) [KDBFD] was an innovative product packaged in the form of sachets. It was based on two classical, popularly used formulae for treating influenza-like diseases known as Wan Bin (溫病). The formula was a combination of Sang Ju Yin (桑菊飲) and Yu Ping Feng San (玉屏風散) plus two other herbs with well-known antiviral properties. The herbs in the formula were Folium Mori (3.75 g), Flos Chrysanthemi (1.5 g), Semen Armeniacae Amarum (3.0 g), Fructus Forsythiae (2.5 g), Herba Menthae (1.25 g), Radix Platycodonis (3.0 g), Radix Glycyrrhizae (1.25 g), Rhizoma Phragmitis (3.0 g), Radix Scutellariae (6.0 g), Folium Isatidis (8.0 g), Radix Astragali (7.5 g) and Radix Saposhnikovia (5.0 g). These raw herbs were boiled to form a decoction and then freeze-dried into granules and packaged (4.0 g per sachet), ready for reconstitution as a tea-like drink. The granules were prepared according to standard Good Manufacturing Practice.

In addition to KDBFD, four traditional Chinese medicines (TCMs) were included in the present study: *Houttuynia cordata* (HC), which has been used to relieve lung-related symptoms and its anti-inflammatory and anti-viral effects are supported by scientific data; *Sinomenium acutum* (SA), which has been shown to possess anti-inflammatory effects and has been used to treat rheumatoid arthritis in China for over 2000 years; *Coriolus versicolor* (CV) and *Ganoderma lucidum* (GL), which are well known for their immunomodulatory actions.

## Methods

This study was conducted from May 2005 to May 2007 and comprised two clinical trials. The first was a self-controlled study and the second was a randomised, double-blinded, placebo-controlled study. Only the results of the second study are reported. The results of the first study as well as the immunomodulatory and anti-SARS activities of HC have been published elsewhere.<sup>1,2</sup>

Volunteers were screened to ensure they were healthy, based on results of their complete blood count, levels of lactate dehydrogenase and creatine kinase, renal function and liver function. A total of 80 healthy subjects were enrolled. Each eligible subject was randomised to receive placebo or KDBFD treatment, according to a computer-generated code list. Neither the investigators nor subjects knew the treatments assigned. The placebo was prepared to appear, smell, and taste indistinguishable from the KDBFD, and was devoid of pharmacological activity and toxicity. Subjects were required to take one sachet of KDBFD (4 g) or placebo once per day for 7 days. Blood samples were collected from them on

## Key Messages

1. The herbal formula Kwan Du Bu Fei Dang transiently modulated the human immune system.
2. *Coriolus versicolor* had an immunostimulatory effect in mouse splenic lymphocytes.
3. *Ganoderma lucidum* inhibited SARS-CoV RNA-dependent RNA polymerase.
4. *Houttuynia cordata* possessed both immunostimulatory and anti-viral activities.
5. Kwan Du Bu Fei Dang, *Houttuynia cordata* and *Coriolus versicolor* were essentially non-toxic to laboratory animals after oral administration of 16 g/kg.

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The Chinese University of Hong Kong:  
 Institute of Chinese Medicine / School of Biomedical Sciences  
 KP Fung  
 Institute of Chinese Medicine / Department of Orthopaedic & Traumatology  
 PC Leung  
 School of Biomedical Sciences  
 KWS Tsui, CCD Wan, MYM Waye  
 School of Life Sciences  
 KB Wong, WNS Au  
 Department of Chemical Pathology  
 CK Wong, WKC Lam  
 Institute of Chinese Medicine  
 BSC Lau

RFCID project number: 02040332

Principal applicant and corresponding author:  
 Prof Kwok Pui Fung  
 Institute of Chinese Medicine, Rm E203,  
 Science Centre East Block, The Chinese  
 University of Hong Kong, Shatin, NT,  
 Hong Kong SAR, China  
 Tel: (852) 2609 6873  
 Fax: (852) 2603 5123  
 Email: kpfung@cuhk.edu.hk

days 0, 7 and 21. Changes in immune markers including the *ex vivo* production of cytokines, and the percentage and absolute numbers of CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes, the CD4/CD8 ratio, CD56<sup>+</sup>NK cells, and CD19<sup>+</sup>B-lymphocytes were evaluated.

The immunomodulatory activities and anti-viral effects of KDBFD, HC, SA, GL and CV were investigated. Proliferation of mouse splenic lymphocytes was used as the first-line screening assay to evaluate whether the KDBFD or TCMs possessed immunostimulatory activity. Samples with immunostimulatory activity were further studied for their effects on cytokine production and T cell populations. The effects of the KDBFD or TCMs on two SARS-CoV enzymes, RNA-dependent RNA polymerase and 3C-like protease, were evaluated.

The acute oral toxicity test was carried out according to the Procedures and Methods for Toxicological Assessment on Food Safety - Acute toxicity test (GB15193.3-94) issued by Ministry of Health, People's Republic of China.

## Results

The numbers of T-lymphocytes, CD8<sup>+</sup> suppressor plus cytotoxic T-lymphocytes, CD4<sup>+</sup> helper T-lymphocytes, and CD56<sup>+</sup> NK cells in KDBFD treatment group were significantly elevated at day 7, compared to day 0 (all  $P < 0.05$ ). However, such significant elevations of cell numbers were generally not observed at day 21 compared to day 7 or 0 (all  $P > 0.05$ ). The proportion of NK and NK cell numbers was significantly elevated at day 21, compared to day 0 and 7, and day 0, respectively (all  $P < 0.05$ ). In the placebo group, only the cell number of CD8<sup>+</sup> suppressor plus cytotoxic T-lymphocytes showed a significant decrease from day 0 to day 7 ( $P < 0.05$ ). Liver function and renal function parameters in the two groups were not significantly different throughout the study period.

Regarding the *ex vivo* cytokine production of IL-1 $\beta$ , IL-6, IL-8, IL-12, TNF- $\alpha$  and IL-10, there were no significant differences in the stimulation (%) of cytokines released on day 7 or 21 compared to day 0 in the KDBFD and placebo groups (all  $P > 0.05$ ). Inter-group comparison analysis of *ex vivo* cytokine production and lymphocyte subsets in the two groups showed that the increase in TNF- $\alpha$  production (day 21 and day 7 vs day 0) were significantly higher in the placebo than KDBFD group, but the increase in NK cells (day 21 vs day 7) were significantly higher in the KDBFD group (all  $P < 0.05$ ). However, most of the measured immunological parameters were not significantly different (all  $P > 0.05$ ).

### Immunological assays

Splenic lymphocytes of Balb/c mice were incubated with the KDBFD or TCM extracts at concentrations of 0-400  $\mu\text{g/mL}$  in the presence of polymyxin B sulfate for 48 and 72 hours. Extract of CV was found to significantly

stimulate the proliferation of mouse splenic lymphocytes in a dose-dependent manner, whereas KDBFD and SA were immunosuppressive at high concentrations. In contrast, GL was neither stimulatory nor suppressive. Using flow cytometry, it was found that CV extract increased the proportion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. These data indicated that CV stimulated T cell proliferation *in vitro*. After 48 and 72 hours of treatment, the CV extract increased the levels of IL-2 and IL-10 significantly, even at relatively low concentration, ie 100  $\mu\text{g/mL}$ . Moreover, it stimulated the secretion of IL-4 and IFN- $\gamma$  to a certain extent.

### Anti-viral assays

Extracts of KDBFD/TCM inhibited SARS-CoV RNA-dependent RNA polymerase in a dose-dependent manner and their effectiveness in descending order was: GL>CV>SA>HC>KDBFD. Their IC<sub>50</sub> values were 41.9, 108.4, 198.6, 251.1 and 471.3  $\mu\text{g/mL}$ , respectively. In the SARS-CoV 3C-like protease assay, only the HC extract possessed dose-dependent inhibitory activity on this viral enzyme, whilst other TCM extracts/KDBFD showed insignificant effects at doses up to 1000  $\mu\text{g/mL}$ .

### Acute oral toxicity test

There was no significant difference in body weights between KDBFD/HC/CV and the control groups. All live animals appeared normal throughout the 7-day observation period. From our results, KDBFD/HC/CV were essentially non-toxic to laboratory animals following oral administration at 16 g/kg. In contrast, SA and GL caused 70-100% mortality within the same period.

## Discussion

In this study, cell numbers of CD8<sup>+</sup> suppressor plus cytotoxic T-lymphocytes and CD4<sup>+</sup> helper T-lymphocytes were significantly increased after taking KDBFD for 7 days. No such increase was observed at day 21 after KDBFD was stopped as well as in the placebo group. Most immunological parameters in the KDBFD and placebo groups did not differ significantly (all  $P > 0.05$ ). This may have been due to insufficient sample sizes in each group.

The herbal preparation had transient beneficial effect on some immune functions in healthy subjects, but not significant when compared to the placebo group. No adverse event was observed during the study period; liver function and renal function remained normal.

During SARS-CoV infection, lymphocytes are the first line of defence. Attrition of these cells may result in a compromised immune response and eventually the development of disease. In SARS patients, lymphopaenia was usually observed during the initial phase of infection and virus-induced apoptosis was considered to be the major cause of lymphopaenia.<sup>3</sup> The results in mouse splenic lymphocytes demonstrated that HC and CV extracts had an

immunostimulatory effect. They were found to stimulate the proliferation of CD4<sup>+</sup> helper T cells and CD8<sup>+</sup> cytotoxic T cells, which may in turn help to prevent the development of lymphopaenia and the pathogenesis of SARS.

SARS-CoV RNA-dependent RNA polymerase (RdRp) and SARS-CoV 3C-like protease (3CL<sup>pro</sup>) are two enzymes important in the viral replication processes. RdRp is responsible for both positive and negative strand RNA synthesis. It is the essential enzyme in a replicase complex that contains additional viral and cellular proteins. In contrast, 3CL<sup>pro</sup> is responsible for releasing the key replicative enzymes such as RdRp and helicase from the polyprotein precursors.<sup>4</sup> The functional importance of RdRp and 3CL<sup>pro</sup> in the life cycle of SARS-CoV make them the key targets for the development of drugs directed against the virus.<sup>5</sup> From our results, GL and HC were the most potent TCM agents in inhibiting SARS-CoV RdRp and 3CL<sup>pro</sup>, respectively. They may be able to slow down viral growth and minimise its destructiveness.

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