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A prospective study of *Agaricus Blazei* Mycelia Compound administration in asymptomatic HIV-1 infected patients in Lusaka, Zambia

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Running title: *Agaricus Blazei* administration to patients with HIV

Abstract

Agaricus blazei mycelia compound (ABPC®) has been used as a food supplement in Japan for several years. It has been shown to have immune enhancing activities both *in vitro* and *in vivo,* leading us to speculate a potential role in the treatment of HIV infection. To determine the value of ABPC as an immune enhancing supplement for HIV-infected individuals, we carried out a prospective randomized clinical study. We recruited 54 HIV-1 infected asymptomatic patients attending a general clinic in Lusaka, Zambia. Informed consent was obtained from all participants after explaining the purpose and details of the study. At recruitment, all patients had blood CD4 counts >200 cells/mðl. The study participants were divided into the ABPC group (28 subjects) and placebo group (26 subjects). ABPC or placebo was administered by directly observed therapy methodology. During the 12-month period, 3 and 5 patients died in the ABPC and placebo groups, respectively. During that time, 9 and 5 patients in the ABPC and the placebo groups, respectively, left the study and returned to their villages. After 12 months, the average CD4 counts in the placebo group had significantly decreased (P=0.018), while the numbers were maintained in the ABPC group. This finding suggests that ABPC administration is beneficial for maintaining CD4 counts in patients with HIV infection, thereby delaying the onset of AIDS.

Introduction

With 40 million patients world wide, human immunodeficiency virus type 1 (HIV-1) is the most serious infectious disease in the world[1]. Transmitted by sexual contact, blood transfusion and drug use involving needles, the virus infects CD4(+) T cells and replicates, with the decline and destruction of these cells compromising the immune function of the host[2]. The infected host cells then release accessory proteins, such as Tat, that decrease the activity of NK cells and Interleukin (IL)-12 production, further weakening the immunity of the host [3, 4, 5]. Therefore, opportunistic infections and cancer often becomes the cause of the death of HIV infected patients. An inversely proportional relationship was discovered between the number of CD4(+) T lymphocytes and the appearance of opportunistic infections: a CD4 count below 200 cells/mðl signals the onset of AIDS even when there are no other symptoms and indicates that anti-HIV treatment must begin [6, 7]. Therefore, maintaining a CD4 count above 200 cells/mðl becomes crucial for preventing the onset of AIDS.

As yet, no effective measure has been established to prevent the onset of AIDS. In the United States, Europe and Japan, a food supplement, *Agaricus blazeri* and its derivatives including ABPC (*Agaricus blazei* mycelia treated with Hemicellulase) are popularly taken by wasting persons or cancer patients for its immune-strengthening properties. Both *in vivo* and *in vitro* animal experiments have demonstrated its benefits as an NK cell activator as well as its capability of activating the immune system [8-15]. Previously, we reported that this ABPC was able to augment the production of IL-12[16]. Furthermore, we were able to confirm that the IL-12 levels were higher in long-term asymptomatic HIV patients than in symptomatic HIV patients (unpublished data). Accordingly, we hypothesized that the long-term administration of ABPC would maintain the CD4 count, and thus delay the onset of AIDS. To confirm this hypothesis, we administered ABPC for 12 months to HIV patients with a CD4 positive cell count above 200 cells/mðl.

Subjects and Methods

Patients

We advertised the study at HIV voluntary counseling and testing centers in Lusaka, Zambia. When patients volunteered to join the study, the details of the study, approved by the ethical committee of the University of Zambia, were explained at a private clinic in Lusaka. The volunteers were then interviewed and examined

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by a physician to determine if they fit the recruitment criteria. The recruitment criteria were 1) no HIV-related symptoms, 2) no clinical or X-ray signs of tuberculosis, 3) a CD4(+) T-cell count higher than 200 cells/mδl in blood at recruitment, 4) no treatment by anti-retroviral drugs, and 5) the participant's agreement to receive home visits by a nurse. After fully explaining the study, informed consent was obtained from all participants.

Directly observed therapy

The study participants were randomly assigned to either 2g ABPC® (Japan Applied Microbiology Research Institute Ltd, Yamanashi, Japan)[16] or 2g dextrin as placebo (Matutani Kagaku Co. Japan) administration. To keep good adherence to supplement administration, ABPC or placebo was taken twice a day by directly observed therapy methodology.

Follow up

Everyday, the participants took the assigned supplement in the presence of a health worker. They were also clinically evaluated by a physician at the beginning and after 2, 6, and 12 months of treatment. At recruitment and after 12 months, 3 ml of peripheral blood was obtained in an EDTA tube from patients for CD4 count and HIV RNA load. When participants did not show up at the clinic to take the supplement, health workers visited the participant's house to ascertain their reason for not coming to the clinic and to evaluate their health condition.

CD4 counts

Whole blood cells were stained with FITC-labeled anti-CD3 mAb (BD PharMingen), PE-labeled anti-CD4 mAbs (BD PharMingen), and isotype controls (BD PharMingen) for 15 min at room temperature. Red blood cells were lysed using red blood cell lysing buffer (BD Biosciences) and washed. The stained cells were then fixed with 2% formaldehyde, and resuspended in FACS buffer. Cells were analyzed for expression of CD3 and CD4 within a lymphocyte gate on a FACSCalibur (BD Biosciences, San Jose, CA). The complete blood count was quantified by an automated full blood counter KX-21 (SYSMEX, Kobe, Japan). The absolute number of CD4(+) T cells in peripheral blood were calculated based on the full blood count and percentage of CD4 positive T lymphocytes.

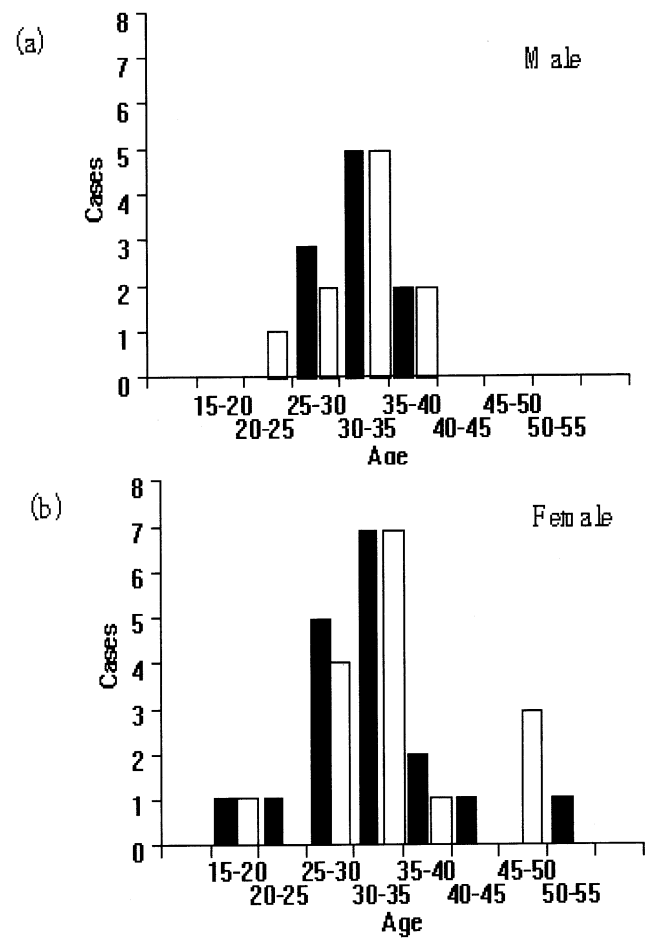
Measurements of Virus

Plasma HIV-1 RNA levels were measured with use of Amplicor HIV-1 Monitor kit (version 1.5, Roche Diagnostics Systems Inc., Branchburg, NJ), according to the manufacturer's instructions. RNA levels were measured on a logarithmic (base 10) scale.

Statistical Analyses

The SPSS software package (version 11.0J for Windows) was used for analyses. Gender distribution between two groups was compared using Fisher's exact probability test. Age, WBC, lymphocyte count, CD4 count and HIV-1 load between groups were compared by the two-sided paired Student's t-test. P-values <0.05 were considered statistically significant.

Figure 1
Distribution of age in (a) male and (b) female participants of this cohort. Closed and open bars show the number of the participants in the ABPC and placebo groups, respectively.



Results

We recruited 54 HIV-1 infected asymptomatic patients who were diagnosed as seropositive at HIV voluntary and counseling testing centers before attending this study. Twenty-eight and 26 participants were randomly assigned to ABPC and placebo administration, respectively. Distribution of age and gender of participants in these groups was shown in figure 1. At recruitment, the average age of participants was 32.2±8.8 (minimum 19, maximum 54) and 32.9±7.7 (minimum 19, maximum 47) years old in the ABPC and placebo groups, respectively. No significant difference was noted between the two groups (Student's t test: p=0.80).

During the 12-month period, 3 (10.7%) patients in the ABPC and 5 (19.2%) in the placebo group died. In the ABPC group 2 and 1 patient died of pneumonia and gastroenteritis, respectively. In the placebo group 2, 2 and 1 patient died of pneumonia, gastroenteritis and malaria, respectively. There was no significant difference in the cause of death between both groups. During that time, 9 (32.1%) in the ABPC and 5 (19.2%) patients in the placebo group left the study and returned to their villages. After 12 months, 32 individuals comprising 16 participants (5 males and 11 females) in ABPC group and 16 cases (2 males and 14 females) in placebo group were being still followed up. There was no significant difference in the gender distribution among these two groups (Fisher's direct p = 0.39).

At recruitment the average WBC, lymphocyte counts, and CD4 counts in the ABPC group were 5.13 x 10^3, 2.00 x 10^3 and 344 cells/mðl. In the placebo group they were 5.39 x 10^3, 2.37 x 10^3 and 454 cells/mðl, respectively. There was no statistically significant difference for each parameter between the 2 groups (p = 0.667, 0.231, and 0.086, respectively). Although there was no significance, the difference in the average of CD4 counts between the 2 groups was large. The patients were randomly assigned to two groups. Patient numbers were also limited. Therefore it was difficult to avoid such difference. Instead of comparing between two groups directly, we separately evaluated the change of CD4 numbers in each groups. In the placebo group, the CD4 counts lowered significantly by 90 cells/mðl from 454±210 cells/mðl at recruitment to 364±189 cells/mðl at 12 months (p=0.018). The degree of CD4 reduction in the placebo group was almost same as ones in previous reports from different cohorts of HIV-1 natural history (17-19). In the ABPC group, the average CD4 counts, however, had reduced from 342±132 cells/mðl at recruitment to 318±172 cells/mðl at 12 months and there was no significant difference of CD4 count both at recruitment and 12 months. Although the average CD4 counts in the placebo group significantly decreased during 1 year, the counts were maintained in the ABPC group.

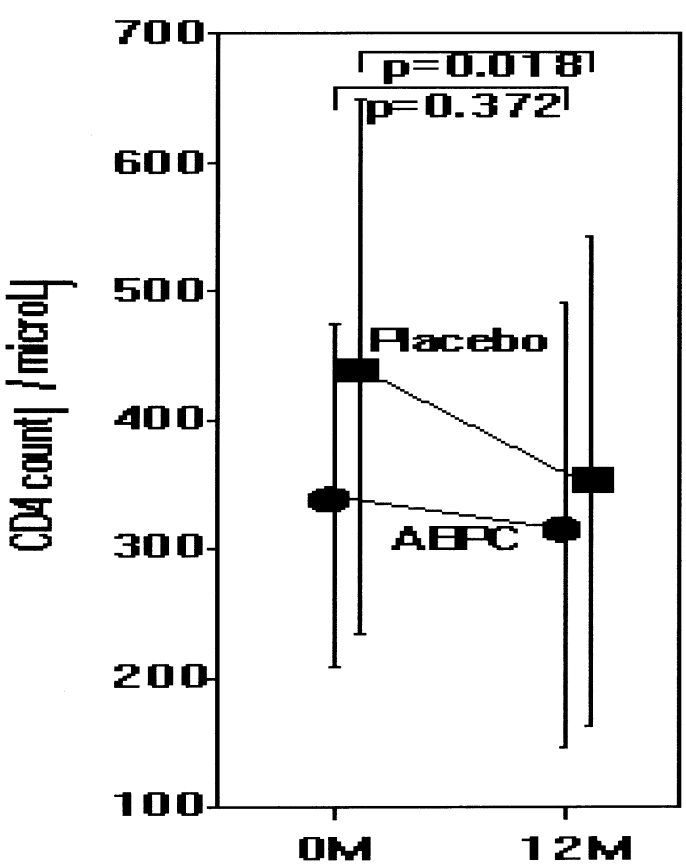


Figure 2

The average CD4(+) T lymphocyte number did not significantly decrease during 12 months in the ABPC group (circles: 16 subjects), but significantly decreased in the placebo group (squares: 16 subjects).

The average of HIV-1 RNA level in patient plasma slightly decreased from 4.45±1.06 $\log_{10}$ to 4.25±1.10 $\log_{10}$ during 12 months in the ABPC group. In the placebo group, it slightly increased from 4.65±1.02 $\log_{10}$ to 4.73±0.99 $\log_{10}$ during 12 months. However, these changes are not statistically significant.

Discussion

To confirm the preventive function of ABPC, we conducted a placebo trial in which ABPC was administered over a 12-month period to HIV infected patients. The placebo group, who were not administered ABPC, showed a significant decrease in CD4 positive cell number, while the ABPC administered group showed no change. Due to the random division of the patients into two groups at the beginning of the study, a difference was found between them. The placebo group showed levels of 454±210 cells/mðl, while the ABPC group had 342±132 cells/mðl at recruitment.

Cohort studies in the United States and Europe reported that asymptomatic patients, with CD4 counts over 200 cells/mðl, showed a 50-120 cells/mðl decrease over a one year period (17-19). Our placebo control group showed comparable numbers with an average decrease of around 90 cells/mðl. The CD4 count levels of the ABPC group, however, significantly showed no decrease. Although there was no suitable explanation for this, previous reports have pointed to the augmentation of IL-12 production and other immune factors through ABPC administration *in vitro*[16]. The key to the maintenance of CD4 levels may lie in this activation of the immune factors such as the raising of IL-12 production in the host.

In the current standard therapy for HIV patients, the initial administration of anti-HIV agent is delayed as long as possible. However, if the CD4 cell count drops

to 200 cells mol. therapy is recommended regardless of the absence of other symptoms. The present therapy of choice is "highly active antiretroviral therapy" (HAART), a combined therapy that inhibits both reverse transcriptase and HIV protease. This therapy helps to return the CD4 cell count to normal and decreases the risk of opportunistic infections. With HAART, it is essential to have at least 95% adherence to administration in order to avoid drug resistance to HIV-1 (20-21). For that reason, it is very difficult to carry out HAART therapy in a developing country such as Zambia. Since ABPC can keep CD4 levels from lowering, it could be a useful for delaying the initiation of HAART therapy.

It would also be worth investigating the possible use of the immune-boosting function of ABPC to allow patients in the middle of HAART treatment to take a break from their treatment. For the first time, we report the benefits of ABPC. in maintaining the immune levels of patients infected with HIV-1. Because of the limited number of patients. the limited follow up period and the difference of initial CD4 count between two groups in this investigation, there is a need for a more detailed study in the future.

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